

UROGENITAL TRICHOMONIASIS  
IN THE MALE.

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## INTRODUCTION

Human genital trichomoniasis was first described in 1868 when the flagellate was found to be present in the urine specimen from a female patient. The first reported infestation of the male was made in 1894.

Most of the investigations into the cause and treatment of trichomoniasis were concerned with the female patient, as the incidence of this infestation in women is high and though the consequences of infestation are not serious in the sense of danger to life; they are serious in that considerable discomfort and mental stress are caused by the presence of *Trichomonas vaginalis* in the genital tract.

A good deal of attention has been paid in recent years to genital trichomoniasis; and more satisfactory techniques for diagnostic procedures, and effective methods of therapy, have been devised. Also, in the past two decades considerable attention has been paid to the problem of trichomoniasis in the human male which has been recognised to be a condition of importance both as a source of infection and re-infection in the female, and as a cause of symptomatic and asymptomatic infection in the male.

## THE PARASITE

The parasite *Trichomonas vaginalis* was discovered by Donne in 1936 in the vagina of certain women suffering from leucorrhoea, and named by him *Trico-monas vaginale*. The name *Trichomonas vaginalis* was given to the organism by Ehrenberg(1838). In 1855 the parasite was found in the vagina of 37 out of 200 pregnant women examined by Kolliker and Scanzoni.

Trichomonads belong to the phylum Protozoa. They are flagellated (class--mastigophora), animal like without chlorophyll (order--zoomastigina) and of the sub-order polymastigina... which comprises such protozoa which are mono-, di-, or multi-nucleate, with three to eight flagella per nucleus and without pseudopodia. The trichomonadidae family are mono-nucleate with an axial organelle and an undulating membrane. The genus *Trichomonas* comprises those with three or four anterior flagella and one posteriorly which forms the undulating membrane.

Trichomonads are very widespread throughout nature. There is almost no limit to their known hosts, which include insects like termites and crickets; fish; many varieties of reptiles; many different birds; and a variety of mammals including man (Trussell, 1947; Hall, 1953).

Most of these various trichomonads are saprophytic; some assist in essential biological processes in the host and some are pathogenic. The most important of the latter, apart from *Trichomonas vaginalis*, are *Trichomonas gallinae*, which affects the anterior digestive tract of pigeons; *Trichomonas gallinorum* which affects the ceca of chickens and turkeys; and *Trichomonas foetus* which affects the genital tract of cattle. The latter, a trichomonad with three flagella, may cause a balanitis in bulls, and pyometra, abortion and sterility in cows. Although it is estimated to affect less than 0.5 per cent of cattle, beasts have on occasion had to be slaughtered because of it. The possible theoretical implications as regards man of an animal genital trichomonad acting in this way are obvious.

The most important trichomonad infesting man is *Trichomonas vaginalis* (syn. *T.vaginale*, *T.vaginae*, *T.irregularis*, *T.vulvo-vaginalis*). Other organisms are *Trichomonas buccalis* which may be found in the mouth; *Trichomonas hominis*, *Trichomonas ardin-delti* (a penta-trichomonad with five flagella) and *T.faecalis*, which are all found in the bowel. Not all authors are agreed as to the separate identities of the bowel trichomonads. Only *Trichomonas vaginalis* is of real clinical importance.

#### MORPHOLOGY.....TRICHOMONAS VAGINALIS

*Trichomonas vaginalis* is a pear shaped, ovoid, or round protozoan 10 - 20 microns in length by 8 - 10 microns broad; with a large nucleus, the macro- or tropho-nucleus close to its anterior end. This macro-nucleus is oval and has a saccular appearance. Adjacent to it is a collection of minute bodies so closely aggregated as to look like a single body very much smaller than the macro-nucleus. This aggregation stains in a manner very similar to the macro-nucleus and is called by some the micro-nucleus or kineto-nucleus; the more correct name is the blepharo-plast. It is connected to the larger nucleus by a very delicate and very short fibre known as the rhizoplast.

Extending outwards from the blepharo-plast are four long hair like structures capable of active movements and known as flagella. Each flagellum has a central fibre or axoneme, covered by a very thin layer of the substance which makes up the mass of the body of the organism. If fresh preparations are observed under dark field illumination with interposed polarising filters



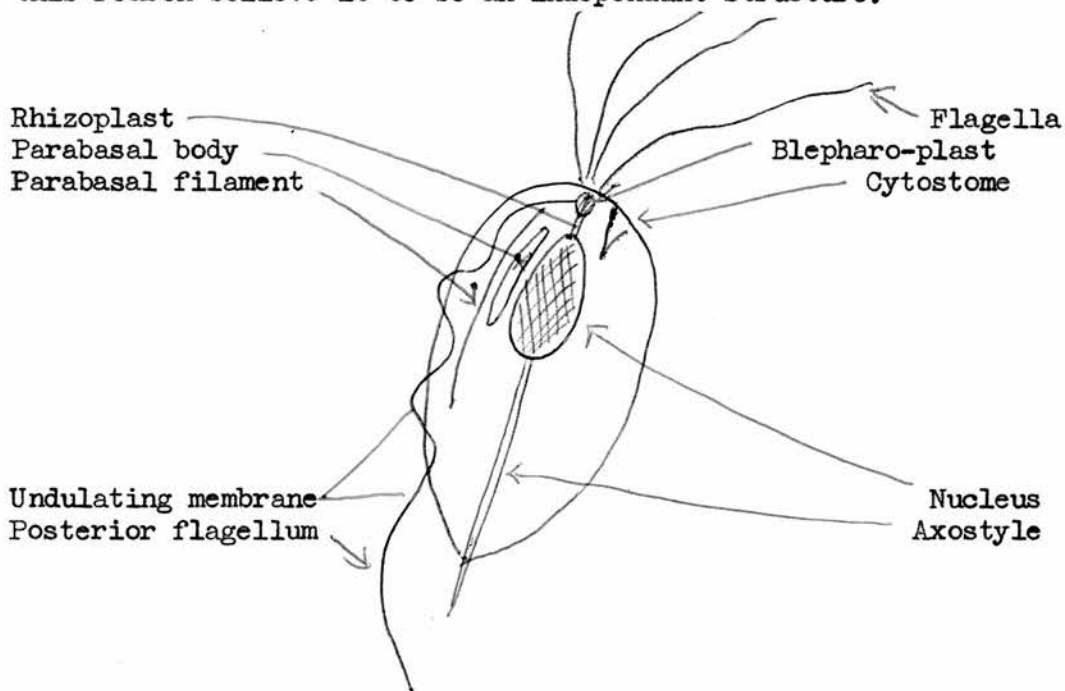
(Silva-Inzunza technique), the flagella appear coloured red.

Feinberg (1954) observed in three different strains of *T.vaginalis* from culture, a morphological characteristic not previously described. This takes the form of a variable thickness and rigidity of the terminal one fourth to one third of one of the four anterior flagella. The presence and conspicuousness of this club like structure varies between strains and between individuals in the ~~same~~ strain. Between individuals, the characteristic varies in conspicuousness from a well defined rod, about two to three times the diameter of the ordinary flagellum, down to a thin structure which is the same diameter as the rest of the flagellum and is manifested solely by its inflexibility. Characteristically a single clubbed flagellum is found on an organism. In many individuals it is absent or undetectable. It is most striking in dark field preparations in which the clubbed portion of the flagellum appears brightly luminous. Feinberg observed the same clubbed structure in organisms in a fresh vaginal specimen. The structure is not therefore a variation arising in artificial culture, but is an intrinsic, if variable, morphological character of *T.vaginalis*.

Feinberg suggests that the function of the club like flagellar structure is open to conjecture. Thickening of the tip of a flagellum has been noted in *Leptomonas ctenocephali*, in which the enlargement is slimy in character and serves as an organ of attachment. The flagellar club of *T.vaginalis*, however, does not appear to be slimy or sticky. The work of Christian et al. (1963) showed at one stage that *T.vaginalis* attached itself to cells by

its axostyle.

Each axoneme arises from the blepharo-plast. The flagella vary in number in different species and generally extend freely outwards from the body in an anterior direction. One axoneme, however, is more closely bound down to the body, sometimes throughout its whole length, by a layer of the material derived from the body, which covers the axoneme. This layer is pulled or stretched out in the form of a thin membrane co-terminus with the body of the organism from the blepharo-plast anteriorly to a distance posteriorly which varies in different species. The axoneme runs along the outer edge of the membrane, and it can communicate to the membrane a wave like motion, hence the name, undulating membrane. Under dark field illumination with polarising filters the undulating membrane is colourless and Coutts et al. (1955) for this reason believe it to be an independant structure.



Within the body of the organism extending from the nucleus is a rod like structure which can be protruded or retracted from

the posterior end of the body; this organ is called the axostyle. When the axostyle is protruded the trichomonas can fix itself to any structure by means of a sticky fluid discharged from its point. When the organism is thus anchored the rhythmic or flail like movements of its flagella, and the sinuous movements of its undulating membrane generate currents in the liquid medium in which the organism generally lies, and by this means particles of food are drawn towards its body. This attachment and whip like movements of the flagella were readily demonstrated in the tissue culture experiments of Christian et al. (1963).

When the organism is not fixed by its axostyle it can swim in a liquid medium by means of its flagella and undulating membrane. If the liquid becomes thickened or clogged with cells the trichomonas can make its way through and among the cells by amoeboid movements. A trichomonas can by this means penetrate the epithelium of a mucous membrane.

Some workers have stated that *T.vaginalis* is furnished with a mouth. Liston (1940) stated that food is taken into the body much after the manner of an amoeba. Presence of glycogen in the vaginal epithelium seems to be necessary for the development of the parasite in the vagina (Liston, 1940); perhaps the organism feeds on glycogen or the glycogen is required to maintain a certain degree of acidity of the vaginal contents favourable for the development of the organism. A film from the vagina of a patient suffering from trichomonas vaginitis may show many of the epithelial cells deprived of their glycogen and evidence that the trichomonads were feeding on it by the presence in them of minute granules of

glycogen stained red by carmine (Liston, 1940).

Inoki et al. (1959) reported from electron microscopic observation that the nuclear membrane was formed of two distinct layers with a discontinuous profile suggesting the presence of pores in the membrane. The periphery of the cytoplasm forms a distinct cell wall or periplast. Several kinds of structures were clearly distinguished within the cytoplasm; round granules 5 to 12 milli-microns in diameter are usually observed, some dense and some less dense. The Golgi apparatus near the nucleus, and vacuoles of different sizes were also seen. Collagen fibrils were very clear and they are likely to connect the base of the flagellum with the surface of the body. Mitochondria were not found and this is supported by biochemical results. An endoplasmic reticulum of the type reported by Palade was seen. Small granules and stripes were observed in an oblique section of the axostyle. The flagellum consisted of an axoneme and covering sheath. Ten pairs of fibrils, one central and nine peripheral, were contained in the axoneme.

Small unflagellated trophozoites have been observed, these may be degenerate forms which recover their properties when they change habitat. Wenyon (1926) and Coutts et al. (1955) considered these to be evolutive forms of the life cycle. Coutts and Silva-Inzunza (1954) claimed that there are occasions when non-motile evolutive forms predominate and thus actively motile forms may not be seen in wet preparations, but with their vital staining technique using fluorescein, evolutive forms and trophozoites are stained and not other cellular elements. Petcherskiy (1951)

claimed that 30 per cent of *T.vaginalis* were rounded in shape, tail-less and motionless.

Fruhwald (1957) stated that rounded forms are more often seen in the male, and more rarely the pear shaped flagellated form. Keutel (1958) also stated that aflagellated forms occur in dense groups in men. Malinovskii (1960) considered that motile trichomonads in the urethral discharge of males were observed only in the late stages of the disease, and in the early stages of trichomonad urethritis no motile trichomonads can be found and the diagnosis must be made by finding morphologically changed non-motile trichomonads. Mesinev (1960) agreed that non-motile forms occur frequently in males.

Teokharov (1958) stated that re-seeding of old cultures of *T.vaginalis* in which non-flagellated structures were present did not produce sub-cultures, and experimental infection of white mice with non-flagellated structures did not result in their infection. He considered that the non-flagellated structures are not cysts but dying or disintegrating trichomonads.

#### REPRODUCTION

Little is known about the multiplication and development of *Trichomonas vaginalis*. Multiplication may be by simple binary fission or by budding. Cysts and pseudo-cysts have also been described. Lydon (1945) described a process which he called "budding", where one large organism (parent cell) was attached to a much smaller one (daughter cell) by a bridge of clear cytoplasm, the outer covering of both organisms being continuous. Separation

resulted by the stretching and final rupture of this bridge owing to the action of the flagella of each organism in opposite directions. After separation each daughter cell was morphologically identical with the parent, though much smaller in size ( 6 - 8 microns ).

Brief mention of division was made by Reuling (1921), who saw division and migration of the blepharo-plast followed by constriction of the cytoplasm to form two daughter cells. Lynch (1922) described division as mitotic in character. He noted that before division the nucleus became larger and less compact and the chromatin separated into scattered particles. The nuclear membrane became more distinct and some spindle formations were seen. After nuclear division, the cell assumed a broad flattened appearance becoming shield-shaped with flagella at the two anterior corners. The cell then split down the middle, each half possessing complete organisation. According to Powell (1936) four chromosomes appear during the mitotic division. Two flagella go to each daughter cell and two new flagella grow out of the blepharo-plast. The old undulating membrane, chromatic basal rod, and parabasal fibril go with one daughter blepharo-plast and similar structures grow out anew from the other.

Multiple fission forms have been described. Marchand (1894) was the first to depict such a specimen. He observed a very large trichomonad containing four nuclei, with flagellar tufts associated with three. Powell (1936) pictures an organism with two nuclei and four sets of flagellar apparatus. Specimens, in cultures, containing as many as eight nuclei have been seen by Trussell (1947).

Kozin (1959) by intra-vital examination of *T.vaginalis* using phase contrast microscopy established that the method of reproduction of the organism varied with the surrounding medium. In favourable conditions direct transverse and longitudinal division predominates, with longitudinal cleavage of the blepharoplast, flagella, nucleus, and membrane. If conditions are unfavourable a schizogony type of reproduction is observed; the organism sheds flagella, becomes rounded and turns into a non-motile cyst. A mixed type of reproduction was also noted.

Lydon (1945) reported that in some cases active organisms disappeared completely from the discharge for periods varying from about one week to two months. During this period, however, cells with a "hard" limiting membrane could always be found on dark field examination, which suggested that the organisms had entered an encysted stage. Wenyon (1926) definitely stated that encystment occurred. Marshall (1954) believed that somewhere in the life cycle of the trichomonas there is a spore or encystment stage. Siebenthal (1945) writes of an encysted form which he says is present particularly in patients who are resistant to treatment. Donald (1952) also believed in the existence of an encysted form which can remain dormant until conditions are more favourable.

Shinozuka (1955) studied the morphology of *T.vaginalis* in culture media under variations of temperature and pH, and under the effect of trichomonocidal agents. In unfavourable conditions the trichomonas becomes a ball shaped organism or "cyst". The "cyst" is capable of becoming an active flagellate when replaced in a favourable environment.



Zinser (1941) from his studies of vital stained and fixed smear preparations of vaginal discharge, concluded that vegetative forms of *T.vaginalis* develop from cysts, later become amoeboid, and finally become encysted again. Schmidt and Kamniker (1926) failed to demonstrate cysts in unstained discharge, but claimed that by using Gram's stain they could detect cysts which were dark with a pale nucleus, though the flagella, and sometimes the undulating membrane, were missing.

Mascall (1954) has observed dying or supposedly dying trichomonads in culture, and observed how the protozoon became practically circular in shape, and the flagella, which finally disappeared, tended to wrap themselves around the protoplasm, which by this time had become granular, to form a type of cyst similar to that described in the male by Lydon (1945). Lanceley (1954) considered that this also occurred under adverse conditions. Bensen (1910), Barlow (1916), Hantke (1921), and Balkow (1935) also described cyst formation.

Trussell (1947) wrote "one also sees motionless, rounded, hyaline forms with no apparent flagella. These are designated as pseudocysts which ultimately disappear and cannot be subcultured". Pātīlā (1948) described in cultures of *T.vaginalis* an immobile round form of size 5 to 6 microns, as well as mobile forms. Davis and Grand (1952) noted that under unfavourable conditions *T.vaginalis* appear as spheres. This was noted in old cultures where the pH had dropped below 5. It was also noted if the culture tube was placed in the refrigerator overnight, but normal shape was resumed when the temperature was gradually raised. Clumping was also observed.



Lejman and Boğdaszewska-Czabanowska (1961) searched for cyst formation and multi-division phenomena of trichomonas with negative results. Only bi-division of the flagellate was noted. Occasionally relatively large forms of trichomonas were observed in wet preparations among living parasites and in stained smears resembling to some degree Lapierre's drawing of cysts (Lapierre, 1957). The authors consider these represent initial stages before bi-division. In some of their cases big groups of trichomonads were seen to be closely packed together. One patient showed these groups in the para-urethral ducts. This phenomenon, the authors considered to be the result of multiplication of the parasites in urethral diverticula, and not washed off by the urinary stream. However, spontaneous grouping of the flagellates does occur, as was shown in the tissue culture experiments of Christian et al. (1963), where the authors termed it "swarming".

The well known susceptibility of *T.vaginalis* to drying suggests that no spore stage exists. Whittington (1957) showed from her experiments with W.C. seats that positive cultures were never obtained from dried material.

Thus, opinions differ greatly as to whether there is a stage of encystment or spore formation in the life cycle of the trichomonad, and the occurrence of rounded, motionless, hyaline forms, apparently without flagella, lends support to this view although unequivocal proof is still lacking. Such spore formation would explain both dormant infection and the resistance to treatment which was an outstanding feature of the condition in the female.

### VIABILITY

The survival of *T.vaginalis* is dependant on many factors, especially those involving associated bacteria, type of medium, temperature, and dessication.

Generally speaking, the more luxuriant the bacterial growth, the shorter lived the protozoan (Trussell, 1947). In bacteria free culture the trichomonad will survive for several days.

Rodecourt (1934) claimed that *T.vaginalis* had remarkable resistance to drying, but Fischer (1935) was unable to confirm this. Vazquez-Colet and Tubanqui (1936) reported that *T.vaginalis* would survive in semi-dry state for at least 6 hours.

The trichomonas is sensitive to variations in temperature; Dock (1896) reported the adverse influence of cold, and noted that urinary trichomonads were not only sensitive to cold but died in 6 - 7 hours at incubator temperatures. Fukushima (1934) kept *T.vaginalis* in an icebox and was able to culture the organisms after three days chilling. Christian et al. (1963) found that a high percentage of the organisms (50 - 100 per cent) may be recovered from the frozen state after short periods of storage, but the recovery after storage for one year at minus 60 degrees Centigrade is low (1 per cent).

High temperature is definitely lethal for *T.vaginalis*. Davis (1929) set the thermal death time at 46 degrees C. for ten minutes. Wu (1938) reported survival for five minutes at 45 deg. but death at 50 deg.C.

The *T.vaginalis* is quite resistant to Ultra-violet radiation and nore so to X-Ray (Trussell, 1947). Teokharov (1958) stated

that *T.vaginalis* perish immediately in 2 per cent soap solution, and remain viable for up to 18 hours in urine.

Putilin et al. (1958) investigated the effect of ultrasonics on trichomonads, using a piezo-quartz generator of frequency of 5.74 megacycles. The trichomonads, placed in an 0.65 per cent solution of Sodium Chloride, died under the influence of the ultrasonic waves after 20 minutes and after 30 minutes they disintegrated.

### BIOCHEMISTRY

Trussell and Johnson (1941) studied the fermentation activities of *T.vaginalis* on various carbohydrates and related compounds. It was found that only glucose and its polymers; maltose, soluble starch, glycogen, and dextrin, were utilised to any appreciable extent. These reactions differ from those of *T.foetus* and differentiate these two trichomonads.

Kupferberg et al. (1948) showed that Pantothenic acid was essential for the growth of *T.vaginalis*. Further studies of Johnson and Kupferberg (1948) demonstrated that certain of the pantothenate analogs possessed potent trichomonocidal activity; but in vivo, however, these compounds were found to be ineffective.

A prime consideration in the study of the metabolism of *T.vaginalis* is the fact that the organism is an anaerobe, and therefore the oxygen concentration must be low if the organism is to survive. Characteristically many anaerobes do not have a cytochrome system, but instead flavine nucleotides are present, which function in the transport of oxygen. Kupferberg was not able to detect the

detect the presence of any cytochrome C in *T.vaginalis*. But using extraction procedures and paper chromatography, both riboflavin and flavin mononucleotide were isolated from the cell. The concentration of riboflavin was found to be 75 mgm/Gm. of cell dry weight; which is a level similar to that found in bacteria.

*T.vaginalis* with all its requirements for a reduced redox potential, has been reported by Kupferberg et al. (1953), Read and Rothman (1955), and Wirtschafter et al. (1956) as taking up oxygen when manumatic experiments are conducted with heavy cell suspensions or cell extracts. Such a phenomenon seems to contradict the basic nature of the organism. Kupferberg (1959) investigated the presence of a DPNH oxidase system in *T.vaginalis*. Such a system is capable of oxidising the niacin containing co-enzyme, DPNH, directly, and the oxygen uptake was found to bear a direct relationship to the amount of the DPNH substrate present. But Kupferberg could not make a definite statement as to whether the oxidation resulted from a single enzyme or group of enzymes.

Metabolism of glucose was also investigated by Kupferberg (1959). Kupferberg et al. (1953), and Read and Rothman (1955) referred to the high concentration of Lactic acid produced by *T.vaginalis* and chromatograms of small samples of growth medium after extraction with ether revealed the presence of no other organic acid. In large volumes of medium, extracted as described by Kupferberg et al. (1953) and examined by paper chromatography, three acids were detected; lactic, malic, and a third, as yet unidentified. The mechanism of the breakdown of glucose to large concentrations of Lactic acid have been reported on by

Wirtschafter and Jahn (1956).

These reports demonstrate that *T.vaginalis* seems to follow the classical Embden-Meyerhof scheme for the degradation of glucose through the various phosphorylated intermediates ending with pyruvate which is subsequently converted to lactic acid.

Kupferberg (1959) also examined carbon dioxide fixation. This phenomenon, once thought to be the exclusive property of photosynthetic systems, is now known to exist in protozoa as well as bacteria. In 1953 Kupferberg reported on the fixation of radio-active carbon dioxide by *T.vaginalis*. Since that time he has identified the end product of this fixation as radio-active lactic acid; and all the radio-activity was confined to the carboxyl carbon of this compound. The specific mechanism involved in the fixation has not yet been demonstrated. The usual malic acid dehydrogenase and carboxylase systems do not seem likely as no labelled malic acid was isolated from the system and cell extracts tested gave no indication of carboxylase activity. The almost exclusive labelling of lactic acid in the carboxyl carbon suggests a mechanism involving entry into the Embden-Meyerhof system. Kupferberg (1959) suggests that the ribulose-1-5-diphosphate-carbon dioxide fixation mediated by a carboxylation enzyme is offered as a possibility.

The majority, but not all, workers, e.g. Mascall (1954), are agreed that the various trichomonads affecting man are of a separate species. There are a number of differences including morphology. *Trichomonas vaginalis*, for example, had four anterior flagella, the undulating membrane extends for only two thirds of the length of the body and there is no trailing flagellum. *T.hominis* however, often has five anterior flagella (as has *Penta-trichomonas ardindeftei*) and there is a trailing posterior flagellum; *T.buccalis* resembles *T.vaginalis* as regards the flagella and undulating membrane but is smaller in size.

Those who consider that the human trichomonads were of the same species suggested that :-

a. Even in the same site, morphological differences such as size and morphology may be encountered.

b. Such differences are fostered by the environment, (i.e. they wax fat in the vagina because of the better food supply)

Viability also varies. *T.vaginalis* will survive but not multiply at room temperatures, while the others will do both. *T.hominis* can survive in media without serum, but not the others. *T.vaginalis* will live for only half an hour in faeces, while *T.buccalis* and *T.hominis* will survive for 7 and 24 hours respectively, (Trussell, 1947). While *T.hominis* can infect cats, rats, mice, etc., in addition to man; *T.vaginalis* will produce only an asymptomatic infestation of monkeys (Johnson et al., 1950). Moreover while *T.vaginalis* may be successfully inoculated into the vagina of women, *T.hominis* and *T.buccalis* have only been able to

survive for periods of hours to days (Trussell, 1947). Wu (1938) showed that *T.hominis* and *T.buccalis* will survive a temperature of 50 deg. C. for five minutes, but *T.vaginalis* is killed by this temperature.

Inneculation experiments favour the individuality of the different trichomonads. Westphal (1936), for example, studied his own stools and saliva and found no trichomonads over a three year period. He then innoculated his mouth and swallowed *T.vaginalis*, without establishing the parasite in either site. He then swallowed *T.buccalis* and it did not survive in the bowel, although he successfully established the trichomonad in his mouth for a period of ten months. Finally he swallowed *T.hominis* and recovered the same from his stools over a period of from three days to five months.

Finally, serological reactions, using agglutination and complement-fixation techniques and fluorescein tagged antibody techniques, (can be devised to show differences; although there is considerable overlap in these. Serological tests by cross agglutination (Lanceley, 1958), and by fluorescein tagged antibody techniques (McEntegart et al., 1958) suggest that different strains of *T.vaginalis* may exist in man.

If distinct strains of *Trichomonas vaginalis* existed and could be identified they might well be of clinical interest and have an important bearing on both diagnosis and treatment. Unlike bacteria, protozoa are poor antigens and the presence of normal agglutinins in the sera of both man and most domestic animals can be most misleading. Weld and Kean (1958) found that human serum had the capacity to kill and disintegrate

had the capacity to kill and disintegrate *T.vaginalis* with great rapidity. This activity is destroyed by heating to 56 deg. C. for 30 minutes.

The first serological observations on *T.vaginalis* appear to have been made by Reidmüller (1932), who noticed the formation of complement-fixing antibodies in guinea pigs after intra-peritoneal injections of vaginal discharge containing the organisms. Later Tokura (1935) reported the finding of agglutinating antibodies in rabbits after repeated intravenous injections of a killed culture of the organism. However the bulk of the serological investigation of trichomonads had, until the past few years, been carried out by veterinary workers in their study of *Trichomonas foetus*. Of special interest was the finding of the Manley and Belfast strains of *T.foetus* in 1945. Witte (1934) described serum agglutinin formation in bovine trichomoniasis and then Robertson (1941) developed a micro-agglutination technique using sera from artificially immunised rabbits. Trussell (1946) employing the same techniques with *T.vaginalis* obtained satisfactory agglutination reactions.

MacDonald and Tatum (1948) concluded that *T.foetus* was antigenically related to, but distinct from *T.vaginalis* and *T.hominis*; which they considered to be antigenically identical. Menolasino and Hartman (1954) compared *T.vaginalis* with *T.foetus* and concluded that there was antigenic similarity, but McEntegart (1956) compared *T.vaginalis* with two strains of *T.foetus* (Manley and Belfast strains) and showed by agglutination reactions that the three strains were serologically distinct.



Schoenherr (1956) attempted serological differentiation of various trichomonads in animals and man by means of precipitation reactions, complement-fixation, and agglutination of fragmented cells. He found that the complement-fixation reaction was of little value since it gave largely non-specific results. The precipitation test enabled the author to differentiate the various trichomonads from each other. *T.vaginalis* showed two different serological types.

Lanceley (1958) investigated the possibility of strain variations in *T.vaginalis* in Liverpool. Twenty specimens of *T.vaginalis* were tested against an immune serum and 18 specimens were agglutinated. Of ten specimens of *T.vaginalis* tested against two specimens of immune serum, six were agglutinated by one serum and four by the other. No specimen was agglutinated by both sera.

The search for serological variants is handicapped by lack of a suitable test directly applicable to vaginal discharge. Using agglutination methods all strains must be well established in culture before any test is possible. Direct typing methods applicable to fixed vaginal smears or to the scanty organisms from early culture would be invaluable.

McEntegart et al. (1958) investigated the possibility of there being different sero-types of *T.vaginalis* using the method of Coons (1956), which uses the specific staining ability of antibody labelled with fluorescein, and demonstrated differences in fluorescence between *T.foetus* and *T.vaginalis*; and also showed that Lanceley's "Liverpool 52" strain fluoresced less brightly than the original Liverpool strain and this suggests that these

may be serologically distinct varieties. If Lanceley's strain does prove to be a distinct variety it would suggest that the fluorescent method may be a quick and convenient method of investigating the distribution of this and other possible sero-types.

Such an antibody has the property of coating the corresponding antigen, which can be identified by ultra-violet fluorescent microscopy. Anti-trichomonas vaginalis serum is prepared from rabbits (McEntegart, 1956), the globulin fraction of the serum being separated and conjugated to fluorescein isocyanate, the conjugate being subsequently purified by repeated ethanol fractionation and absorption twice with dried liver powder. The final conjugated serum is applied to acetone fixed smears of *T.vaginalis* from pure culture.

#### PATHOGENICITY

While Donne in 1836 described *Trichomonas vaginalis* in certain women with leucorrhoea, it was not until 1916 that Hoehne suggested that this protozoan organism might be of pathogenic significance. In 1940 Trussell and Plass definitely established that *T.vaginalis* can produce the clinical syndrome of *T.vaginalis* vaginitis. These investigators were able to induce the disease in 9 uninfected women by inoculation with a bacteria free culture. This work was later confirmed by Hesseltine et al. (1942). Stabler, Feo and Rakoff (1941) inoculated cultures of the intestinal trichomonad, *T.hominis*, intravaginally. Fifty patients were inoculated, 151 inoculations being made in all. Not only were

these workers unable to produce vaginitis, but they also demonstrated that the protozoa could not even be recovered after a short interval. Subsequent vaginal inoculations with *T.vaginalis* produced a typical infection in 8 out of 10 of these patients.

There have been diverse views as to the potential seriousness of *T.vaginalis* vaginitis. Bland et al. (1931) maintained that it increases puerperal morbidity; while Moench (1939) denied this. Hees (1936) reported that this protozoan organism had been demonstrated by culture and smear in the blood stream, endometrium, chronically inflamed tubes, ovarian cysts, and peritoneum. Also, he stated that pelvic abscess caused by this organism is not rare.

Jacoby and Der Brucke (1935) considered that the trichomonas is a non-pathogenic invader of the normal vagina or of an already diseased vaginal tract. While the presence of *T.vaginalis* in the vagina of a woman is generally associated with symptoms; some women may be infected, but unaware of this. This led to the view that the organism has no pathogenic properties but is a saprophyte. It was also suggested that the absence of symptoms in certain cases may be attributed to the development of immunity, or that there is lacking in those patients without symptoms some additional element, which if present, could initiate symptoms.

Curtis (1914), Hibbert (1933), and Allen et al. (1935), for example, suggested that this additional factor was most probably a streptococcus, and *T.vaginalis* is necessary for the production of symptoms. Some authors look on the trichomonas as a parasitic invader following primary bacterial infection. Lydon (1945) claimed that this view is supported by the marked number of

organisms found in stained smears from trichomonas urethritis even on the second day of the appearance of the discharge.

Liston and Liston (1939) suggested that a suitable pH of the vaginal contents is important for the development of *T.vaginalis*. Clinical symptoms, in their view, only develop when the parasites have established themselves and are multiplying freely. The optimum pH for the multiplication of *T.vaginalis* ranges around pH 6. When the vaginal contents were at the normal pH of 4 to 5 *Trichomonas* was rarely found; and when it is pH 7 to 8, the pH found in children and old persons, trichomonas again ~~the~~ does not develop. Similarly the exacerbation of symptoms immediately before or after the menstrual period can be explained on the grounds of changes in the pH reaction of the vaginal contents. The urethra is often invaded by the trichomonas, and this accounts for the dysuria and frequency common in association with trichomonas vaginitis, here the pH is about 6.

Lewis and Carroll (1928) quote the case of a female patient where trichomonads were found in the urine collected from each kidney; they concluded "the definite symptom complex presented in this patient and the recovery from symptoms coincident with the destruction of the organisms when found in the urinary tract is pathogenic". Kean and Wolinska (1956) studied the cytology of urethral trichomoniasis in women and found acidophilia of the epithelial cells in contrast with the normal basophilia, and increase in polymorphonuclear neutrophils; which led them to suggest that *T.vaginalis* may act as a pathogen in the urethra.

*T.vaginalis* is often found in considerable numbers in the

absence of clinical symptoms or signs; it then appears to be a harmless commensal or saprophyte. The difference in behaviour has not been satisfactorily explained, though many suggestions have been put forward. Mascall (1957) regarded the difference as being due to a variable body reaction to the same organism; others have suggested that only in symbiosis with certain and, as yet, unidentified bacteria does *T.vaginalis* become pathogenic. A third possibility is of variations, morphological or antigenic, in the organism itself. Brehm et al. (1961) suspected that *T.vaginalis* was a facultative pathogenic organism which sets up an immune reaction after infection, because many women (half in their series) who harbour the organism do not have any vaginitis or discharge. Grimmer (1950) also regarded *T.vaginalis* as a facultative pathogen, the transition from saprophytic to parasitic stage taking place on inflamed epithelium.

Hoffman et al. (1961) attempted to discover whether the prevailing ecological conditions of the bacterial flora of the male urogenital tract had any effect on the susceptibility to infection by *T.vaginalis*; a problem which had also been studied by Kozlowski (1951a), Candiani (1953), Feo et al. (1956a), and Popochristov and Neythcheff (1959). Certain differences were noted in the bacterial flora of the urogenital tract of men infected with *T.vaginalis* compared with that of those who remained unaffected despite exposure to infection. A greater number of strains with antagonistic properties were observed in the bacteria from men infected with *T.vaginalis* than in those from the men who were free from infection. Feo et al. (1956a) however

found the bacteria varied, and considered that they could not be implicated as causative. There was also no difference between flora in cases infected with *T.vaginalis* and those not infected.

The significance of trichomoniasis of the male urogenital tract has been disputed by various authors. This disagreement has arisen from the fact that not all patients whose urinary or prostatic secretion contains the organisms have urological ~~xx~~ symptoms. A number of them do, however, and in the absence of concomitant pathological conditions, elimination of the trichomonads is followed by the disappearance of the patient's complaints. This seems to be particularly true of men whose complaints suggest chronic prostatitis or urethritis, and in whose prostatic smears *T.vaginalis* are found. While the question of the pathogenicity of trichomonads in urine specimens has been doubted, laboratory examinations of the prostatic smears suggests that at least in these instances the organisms are pathogenic, for in addition to trichomonads, such smears invariably contain many pus cells and bacteria, by no means normal smears. Allison (1943) believed that the organisms were frequently pathogenic in the male and pointed out that 95 per cent of over 200 coloured draftees whom he had examined had urethral strictures wherever trichomonas was found.

*T.vaginalis* infestation in the female is relatively common while *T.vaginalis* urethritis in the male is not so common, (though some authors report a very high incidence... vide INCIDENCE ). It has been suggested that either (1) *T.vaginalis* fails in the great majority of cases to bring about a pathological reaction in

exposed males, or, (2) when *T.vaginalis* is discovered in any condition, it is grafted on some other infection which allows for its growth, i.e. simple parasitism. If either or both of these theories were not correct, then not only would all married men whose wives harbour *T.vaginalis* suffer from urethritis, but the condition would already have become so common as to outstrip Gonorrhoea in frequency. Weston and Nicol (1963) suggested that the male may develop some kind of natural resistance or immunity to trichomonal infections.

Further doubt was thrown on the concept of *T.vaginalis* as a parasite of little more than nuisance value by the work of Schnitzer et al. (1950) who showed that mice can be infected with *T.vaginalis* by intra-muscular injection, giving rise to a localised abscess. Inoki and Hamada (1954), in an attempt to produce fatal infection, inoculated mice intra-peritoneally with pure culture of *T.vaginalis* and achieved a mortality rate of 70 per cent. But using 0.05 ml. of peritoneal fluid from a previously infected mouse resulted in a mortality rate of 100 per cent in 3 - 4 days. From this they concluded that animal passage of *T.vaginalis* results in enhancement of pathogenicity. Johnson et al. (1950) inoculated Rhesus monkeys with cultures of *T.vaginalis* and were successful in producing infection but no signs of vaginitis developed; and they noted that the highest numbers of *T.vaginalis* were present in the vagina in the period one week before and after menstruation.

Experiments of Trussell and Plass (1940) in women, and of Lanceley and McEntegart (1953) in men, have established beyond doubt the pathogenicity of *T.vaginalis*. Lanceley and McEntegart



innoculated 5 volunteer patients intra-urethrally with a pure culture of *T.vaginalis*, and 5 with sterile culture medium as a control. No abnormality developed in the controls but three of the infected patients developed signs of urethritis.

A trichomonas can penetrate the epithelium of a mucous membrane, and Wenyon (1926) has shown in sections trichomonads under the epithelium of the bowel. It is probable that the small papules in the vagina, which are so characteristic of trichomonas vaginitis, and which give rise to a sensation of roughness to the examining finger in the vagina, may be caused by penetration of the parasite below the epithelium. Gavrilescu (1960) showed in experimental cultures on Loefflers medium that *T.vaginalis* induces lysis of the medium. When inoculated in tissue cultures, it has a conspicuous toxic or lytic action. The author considers that these proteolytic effects of the parasite explain the erosive lesions in the mucous membrane of the genital tract.

Vassallo (1946) felt almost sure that Abacterial Pyuria was connected with sexual intercourse and that some inhabitant of the vagina, of perhaps low grade toxicity, might find its way into the urethra and set up symptoms. Wheatley (1946) and Fieldsend (1946) favoured the action of trichomonads in Abacterial Pyuria. This theory could explain the known efficacy of intra-venous arsenical preparations in the treatment of this condition. However since the immediate post-war period Abacterial Pyuria seems to have disappeared as a clinical entity and this could hardly be possible if the trichomonad were an aetiological factor.

Pentimali (1925) found *T.vaginalis* in the circulating blood



of humans. Wagner and Hees (1937) cultured *T.vaginalis* from the blood of a woman, and in 75 cases of vaginal trichomoniasis obtained in 53 (75.3 per cent) positive haemo-cultures for *T.vaginalis*.

The effect of *T.vaginalis* on fertility (cf. Bovine trichomoniasis) has been studied by some authors and Allison (1943) claimed that several sterility cases were found to be infected with *T.vaginalis*. Kolesow (1950) in an account of the action of *T.vaginalis* on spermatozoa in vitro, claimed that the spermatozoa first lost their motility and then were ingested by the protozoa; even those that escaped this fate were lysed within about three hours. These observations have not been substantiated yet, but if they were, there might be even further reason to emphasise the importance of male infection with *T.vaginalis*. Keutel (1958) stated that culture experiments appear to show that *T.vaginalis* affect fructose level and fructolysis of spermatozoa, which could perhaps affect fertility. Döring (1962) made the incidental observation that six of the patients in his series who had been infertile for many years became pregnant immediately after treatment of a trichomonas\*infection. Bauer (1963) believes that trichomoniasis may result in sterility in the female.

The pathogenicity of *T.vaginalis*, has I think, been established beyond doubt by the work of Christian et al. (1963). These workers investigated the effect of inoculating *T.vaginalis* cultures into tissue cultures of HeLa cells isolated from carcinoma of the cervix. Two previous investigators had reported experiments in which explants of chick embryo gut cells were

exposed to the action of the flagellates. Houge (1947) felt the cytopathology observed was due to a toxic substance, rather than mechanical damage caused by the parasite; but Kotcher and Hoogasian (1957) were unable to demonstrate a toxin in their *T.vaginalis* cultures for chick embryo explants, HeLa cells, or human synovial cells.

Christian et al. (1963) found that the cell parasite relationship can be divided into four phases :-

1. HeLa cells inoculated with a culture of *T.vaginalis*, and the flagellates can be observed moving freely over the cell sheet.
2. The organisms disappear from view.
3. Small holes or plaques appear in the HeLa cell sheet, their edges lined with trichomonads, attached to the cells by their axostyles and beating the open area with their flagella.
4. The plaques become confluent and most of the culture cells are destroyed.

From their experiments the authors established that *T.vaginalis* will grow, **divide** and destroy HeLa cells in tissue culture medium with the addition of either human or calf serum, but under the same conditions but in the absence of He La cells the trichomonads will exist but not grow. With a small inoculum a stationary phase supervened which suggested a carrier state, which the authors considered might be analogous to the asymptomatic infection common in both the human male and female.

### INCIDENCE

The reported figures for the incidence of *T.vaginalis* infection in the male differ greatly. The figures reported are mainly from Venereal Disease Clinics and therefore represent selected groups comprising mainly men who had either had symptoms or had been promiscuous. This variance in figures quoted for the incidence of trichomoniasis in the male may be because the incidence does, in fact, vary; or because the means of diagnosis are not reliable. The incidence of female infections varies little from country to country, and failure to obtain similar constant figures for male infections is therefore possibly due to defects in laboratory diagnosis; though with the greatly improved cultural techniques now available, the incidence reported in recent series in the United Kingdom is still low, though authors in Europe report a much higher incidence of male trichomoniasis than authors in this country.

It is notable that a high percentage of cases of male infection reported are asymptomatic, e.g. Whittington (1957) 19%, Watt and Jennison (1960) 27%, Ljahovickij (1960) 33.8%, Catterall (1960) 14.3%, and the highest of all Block (1959) 79%. It is therefore likely that there may be large numbers of men, possibly a vast number, with *T.vaginalis* infection who are asymptomatic and therefore have no cause to attend a clinic for diagnosis and treatment. This could constitute a vast reservoir of infection. Another possibility is that in many men the infection is self-limiting, a belief held at one time by many authors, e.g. Karnaky (1938b), Dastidar (1925).

In 1868 Salisbury reported the presence of *Trichomonas vaginalis* in the urine specimen of a woman, which he had examined. Since that time, reports of similar findings in both men and women have accumulated in the literature with gradually increasing frequency. The incidence of such infections in men varies greatly in different reports, from 1 to over 60 per cent of cases of Non-Gonococcal Urethritis; British authors quoting an incidence of 4 - 16 %, and European and American authors reporting a much higher incidence (see Table I, p.32). The incidence of *T.vaginalis* vaginitis in women being much higher.

A paper by Dock (1896) reviewed the available knowledge of the flagellates parasitic in man. He stated that undoubtedly the first case of trichomonas infestation of the urinary tract of a male patient was published by Marchand (1894) .... a man aged 60 had suffered from a fistula in the perineum for 17 years following on pelvic suppuration, and the condition was regarded as tubercular. Pus suddenly appeared in the urine without symptoms of cystitis, and flagellates were found in the urine. Marchand concluded, as a result of comparative examinations of this flagellate parasite from the bladder and flagellates found in the vagina in certain females, that there was a great resemblance, if not identity, between them. However, Künstler (1883) reported a case of *T.vaginalis* urethritis, and the honour of being the first to report this condition should go to this author.

TABLE I

Incidence of *Trichomonas vaginalis* urethritis in males with  
Non-Gonococcal Urethritis

Author	Date	No. of patients in series	% T.V. positive
Ackermann	1935	37	0
Nitschke	1936	40	12.5
Leberman	1937	500	1.0
Knight & Shelanski	1939	124	42.0
Liston & Lees	1940	94	16.0
Allison	1943		15.0
Roth	1944	62 Negro	27.4
		100 White	4.0
Feo	1944	310 Negro	39.0
		80 White	29.0
Freed	1948	112	28.5
Pentinnen & Rauramo	1948	7	42.9
Frühwald	1950		47.2
Kucera	1950	588	6.4
Valina	1950		28.8
Grimmer	1950		21.0
Leca	1951	200	10.0
Kozlowski	1951b		10.0
Kolankowski	1951		15.0
Sorel	1952	236	9.0
Harkness	1953	925	6.4
Seneca & Ides	1953	246	37.0
Lanceley	1953	310	5.8
Ambrose & Taylor	1953	45	2.2
Durel et al.	1954	288	12.15
Lanceley	1954	100	4.0
Shepherd	1954	54	1.9
Coutts et al.	1955	2,482	68.0
Lomuto & Ciaula	1955	137	18.98
Feo et al.	1956b	17 White	10.5
		56 Negro	51.8
Harkness & King	1957		15.3
Teokharov	1956	251	26.3
Frühwald	1957		37.0
Jira	1957	1,961	8.7
Whittington	1957	326	15.3
Doyle et al.	1957	207	0.5
Siboulet	1957	1,097	6.6
Nicol	1958	466 White	3.0
		117 Negro	15.0
Verheye & Kanda	1958	912	15.0
Durel	1959	811	10.4
Jira	1959	1,972	10.0
Sylvestre et al.	1959		11.0
Rosedale	1958	150	1.5
Röckl & Naseman	1959	652	8.0

TABLE I Contd.

Author	Date	No. of patients in series	% T.V. positive
Siboulet	1960	1,333	11.4
Catterall	1960	2,300	5.5
Kupriyanov	1960	86	32.5
Ryigas	1960	59	50.0
Teokharov	1960	276	26.5
Gallivanone	1960		5.7
Lejman et al.	1961	162	12.3
Hoffman et al.	1961	258	7.0
Duncan & Morton	1962	33	12.0
Hoffman & Kilazewski	1962		41.1
Siboulet	1963	808	25.3
Sylvestre & Ethier	1963	40	5.0
Weston & Nicol	1963	1,525	6.09
Nnochiri	1964	100 Negro	8.0

Miura (1894) reported the finding of the flagellates in the urine of a man aged 52. A self-retaining catheter demonstrated that the organisms came from the urethra and not the bladder. The patient's wife had trichomonas vaginitis. Dock (1896) described the case of a young man, who had frequency, dysuria, and haematuria following pneumonia. Flagellates were found in the urine. Katagunuma (1924) reported a case of T.vaginalis infection in a boy aged three years who might have been infected by a female attendant. The flagellates in this case were multiplying in the preputial sac where they caused redness and oedema. This was the first reported case of trichomonas balanitis.

Dastidar (1925) reported the trichomonas in four cases during routine examination of some thousand specimens of urine from both sexes. Three of these cases were males. In each case the urine was acid, contained a few pus cells, and numerous trichomonads. He concluded that the trichomonas may cause a mild

urethritis, but that the urethritis cures itself on the disappearance of the trichomonads.

The first definite account of *T.vaginalis* urethritis in the male was that of Capek (1927) cited by Grimm (1930) and Riba (1931). Capek observed two cases of trichomonas urethritis during the course of one year. One had haematuria with numerous trichomonads in the urethra; his wife had trichomonas vaginitis. The other patient had a severe urethritis with *T.vaginalis* present in the discharge, and also an epididymitis. Lewis and Carroll (1928) noted the presence of trichomonads in the kidney. Riba and Perry (1929) reported two cases of trichomonas infection in males, one of whom had trichomonas in the prostate.

Grimm (1930) described five cases of trichomonas infection in males. Rosenthal (1931) reported a case of trichomonas urinary infection in a man aged 72. The urine was acid, contained pus cells, coliform organisms, and trichomonads. When the urine was rendered alkaline the trichomonads disappeared and symptoms subsided. There was no relapse later when the urine became acid again. St hler (1933) reported that out of 32,000 examinations of prostatic fluid at the Mayo Clinic, *T.vaginalis* was found only 16 times. The technique is not described and the findings are open to criticism. Allen et al. (1935) found trichomonas in the fresh films of the prostatic fluids of 6 out of 9 husbands whose wives suffered from trichomonas vaginitis. Cornell and Riba (1936) stated that in seven years they came across 30 cases of trichomonas infection in men. They believed that trichomoniasis in the male is acquired solely through sexual contact.

The paper by Liston and Lees (1940) was the first paper to be published in Britain on the subject of trichomoniasis in males, and they found *T.vaginalis* in 16 out of 400 males attending a Venereal Diseases Clinic for all conditions, this was 16 % of all cases of Non-Gonococcal Urethritis. Kucera (1950) examined 588 men with urethritis, balanitis, chancre, epididymitis, and orchitis, and found *T.vaginalis* in 38 (6.4 %). Another 31 were probables as atypical forms of *T.vaginalis* were seen and the patients had suggestive histories.

Bauermeister and Hollinger (1941) after a search of world literature discovered records of only 145 cases of infection of the male urethra by *Trichomonas vaginalis*. Since 1940 there have been many articles in medical journals both in Britain and in other countries on the subject of trichomoniasis in the male and these show a wide discrepancy regarding incidence (see Table I, p.32). Popochristov and Berov (1961) stated that the relative percentage of urethritis and vaginitis due to trichomonas rises with increased rates of non-gonococcal infections.

The rise in the incidence of Non-Gonococcal Urethritis during the period of World War II and the continued rise following the immediate post-war drop, stimulated considerable research into the aetiology of Non-Gonococcal Urethritis and thus the trichomonas was found to be a causative organism of some significance. This stimulated further interest in *Trichomonas vaginalis* and a great deal of information has been produced. The resemblance of these organisms, especially in their immobile and atypical forms, to other cellular elements might well be the cause of these



conflicting reports in incidence. Furthermore the belief in an encysted resting phase would tend to produce higher figures for the incidence (vide Coutts et al., 1955).

#### CONSORTS

Examination of husbands and consorts of infected women showed an incidence of *T.vaginalis* infection varying from 4 - 90 per cent, (see Table II, p.37). Many of the husbands and consorts in these series were found to be symptom free, but they were none the less a potential source of re-infection of wives successfully treated. Bedoya and Rios (1958c) found an incidence of 67 per cent *T.vaginalis* infestation in husbands of women with *Trichomonas* vaginitis, but stated that in those cases where *T.vaginalis* could not be found...the past history and symptoms were so suspicious that existence of *T.vaginalis* could be presumed. It was stated at the 1957 Symposium at Rheims that 78 per cent of the male partners of patients having *trichomonas* vaginitis are infected and will remain so until treated.

The examination of wives and consorts of men suffering from *trichomonas* infection showed an incidence of *Trichomonas* vaginitis ranging from 60 - 100 per cent; a much higher degree of infection than in male consorts of infected women (see Table III, p.38).

The examination of wives and consorts of male patients with Non-Gonococcal Urethritis, in whom *T.vaginalis* could not be found, showed a high incidence of *trichomonas* vaginitis (see Table IV, p.38).

TABLE IITrichomoniasis in male consorts of women with *T.vaginalis* vaginitis

Author	Year	No. of consorts examined	% T.V. positive
Stein & Cope	1935	3	33.0
Ackermann	1935	6	0.0
Allen et al.	1935	9	66.0
Drummond	1936	5	80.0
Young	1949		21.2
Whittington	1951a	26	27.0
Bernstine & Rakoff	1953	60	6.6
Kostic	1954	98	34.7
Mascall	1954	150	4.0
Keutel & Neumann	1955	86	97.0
Coutts et al.	1955		40.0
Clifford	1956	7	28.6
Perl et al.	1956	48	58.0
Barnes et al.	1957	8	25.0
Bedoya & Rios	1957b	62	67.0
Jira	1957	31	32.0
Whittington	1957	24	33.3
Bedoya & Rios	1958c	62	67.0
Keutel	1958	152	37.7
Nicol	1958	107	12.1
Verheye & Kanda	1958	128	47.0
Bedoya	1959		76.0
Bertrand & Leulier	1959	27	74.0
Block	1959	148	61.5
Kostic	1959	364	39.0
Kozin	1959		70.0
Perju	1959	100	41.0
Ensey	1959	34	61.8
Netter	1959		90.0
Keutel & Rothe	1960	24	79.2
King	1960	38	29.0
Rees	1960	10	30.0
Scott-Gray	1960	6	50.0
Siboulet	1960		80.0
Watt & Jennison	1960	30	60.0
Brehm et al.	1961		25.0
Hoffman et al.	1961	50	30.0
Dao	1963	85	28.2
Weston & Nicol	1963	206	45.1
Hesseltine & Lefebvre	1963	21	4.76
Crowley	1964	11	100

TABLE III

Wives and consorts examined, of male patients with Trichomoniasis.

Author	Year	No. of consorts examined	% T.V. positive
Nitschke	1936	5	100
Riba & Harrison	1940	14	47.0
Young	1949	17	64.7
Lomuto & Ciaula	1955	26	83.3
Whittington	1957	26	81.0
Nicol	1958	18	83.0
Catterall	1960	67	85.0
Catterall & Nicol	1960 <sup>b</sup>	56	100
Ryigas	1960	8	100
Popochristov & Berov	1961	17	100
Shindelkroit	1961	146	66.4

TABLE IV

Wives and consorts examined, of men suffering from Non-Gonococcal Urethritis.

Author	Year	No. of consorts examined	% T.V. positive
Ackermann	1935		16.0
MacFarlane & Johns	1958	20	15.0
Mayne	1957	18	55.5
Burgess	1959	250	34.8
Rosedale	1959	150	12.0
D			

### AGE INCIDENCE

Young (1949) found no *T.vaginalis* in the urine of 300 male children. Urinary trichomoniasis in children is rare but has been reported on several occasions in girls. Katsunuma (1924) described the organisms in the urine of a boy 3 years old. Strain (1945) reported three cases in boys under 6 years of age. Nicol (1958) stated that the incidence of trichomonas urethritis was higher in the older age groups compared with the incidence of Gonorrhoea.

In women, Perez and Blanchard (1957) found that the great majority of cases occurred between 20 - 40 years of age, i.e. the sexually active period, and cases were rare before 15 and after 60. Married women gave the greatest incidence, but the authors considered that this was not because the condition was rare among unmarried women, but because single women who exposed themselves to possible infection by coitus commonly used a chemical contraceptive, which had a trichomonicidal action.

Crowther (1962) reported two cases of trichomonas infection in female infants, in one the mother had been treated for trichomonas infection in pregnancy, but in the other case the source of infection could not be elicited.

### RACIAL DIFFERENCES

A higher incidence in the negro male than in the white male has been reported by several authors, as shown in Table V, p.40. A similar racial difference is reported in females. Trussell (1947) quoted 23.5 per cent incidence in white women and 45.2 per

cent in coloured women. Burch et al. (1959a) reported 8.1 per cent in white patients and 60.9 per cent in coloured patients. This racial difference might possibly be due to different socio-economic conditions under which the two races live, or to difference in the incidence of promiscuity.

TABLE V

Incidence of *T.vaginalis* infection in males, by race.

Author	Year	% <i>T.vaginalis</i> positive	
		Negro	White
Roth	1944	27.4	4.0
Feo	1944	18.9	12.4
Feo	1953	16.6	12.0
Feo et al.	1956a	51.8	10.5
Whittington	1957	28.4	12.5
Nicol	1958	15.0	3.0
Weston & Nicol	1963	15.0	1.8

ASSOCIATION WITH OTHER INFECTIONS

While trichomoniasis in association with Gonorrhoea in females is a common occurrence, in 25 per cent of cases according to Bogacheva (1958), and 52 per cent according to Malinovskii (1960); the similar association in the male, though it has been reported on a number of occasions, is considered an uncommon one. The rarity of this occurrence might be due to failure to identify the parasite in the "packed field" of gonococcal exudates, but even dilution of the preparation results in no higher incidence of positive results. Though Hoffman et al. (1961) found a higher incidence of trichomoniasis in male patients after the gonorrhoea had been treated than in patients prior to treatment, thus suggesting that the presence of the acute gonococcal infection

tended to "mask" the presence of the trichomonads in some patients.

Lanceley (1953) estimated the pH of gonococcal urethral exudates and the average approxiamted to 6.9 compared with pH 6 for a similar sample of exudates from Non-Gonococcal Urethritis. While it is not completely unsuitable for *T.vaginalis*, a pH of 6.9 is above the optimum for growth of the protozoa in vitro and may be a factor in the infrequency of this double infection in males.

Pray (1952) reported that certain bacteria greatly curtail the multiplication of *T.vaginalis* in vitro, while others have a moderately inhibiting effect. It is possible that the gonococcus has an inhibitory effect on *T.vaginalis*. This would not necessarily apply in the female, as the trichomonas causes a vaginitis mainly, and the gonococcus affects the urethra and cervix, and does not affect the vagina.

TABLE VI

Incidence of concomitant Gonorrhoea and Trichomonas infection.

Author	Year	Patients with Gonorrhoea.	Percentage T.V. positive.
Liston & Lees	1940	264	0.4
Feo	1944	425 Negro	4.2
		111 White	0.9
Lanceley	1953	285	0.7
Feo et al.	1956a	108	1.8
Bogacheva	1958		12.0
Hoffman et al.	1961	Before treatment	
		96	4.1
		After treatment	
		64	6.1

### SEX DIFFERENCES IN INCIDENCE

As has been seen, the reported incidence of trichomonas infection in males with urethritis varies from 4 - 68 %, with the reported incidence in Britain varying from 1.5 - 16 %, whereas the estimated incidence of infection in women is much greater, (8 - 53 per cent of all women; Trussell, 1947).

Nitschke (1936) believed that the difference in incidence between the two sexes is easily accounted for :

1. Anatomically by the close proximity of the rectum and vagina in the female (which pre-supposes infection of the vagina from the rectum, a theory no longer tenable), and the less fertile field of the long tubed urethra of the male.

2. Physiologically by the presence of the urinary stream in the male, mechanically irrigating the urethra.

3. Biologically by the size and delicateness of the organism.

4. Pathologically by the comparative superficiality of the disease process.

5. Widespread presence in the female is not dependant on sex relationship, but possibly occurs by contamination.

Mascall (1954) believed that the lower incidence of trichomonas infection in the male was due to the differences in the hormonal response of the sexes. In the female, oestrogens produce a deposit of glycogen in the vaginal mucosa, and the trichomonas can metabolise glycogen. Also differing reactions of the sexes to certain infections is well known in syphilis; for syphilis in the female takes a much milder course than in the male,

especially so during pregnancy. Thus Mascall considered that if a suppressive factor can work in syphilis in the female, there is no reason why a suppressive factor should not work in trichomonas infection in the male.

Many of the investigations in the last two decades have reported not only urethritis, but prostatitis and epididymitis caused by *T.vaginalis*. The incidence and severity of the infection with *Trichomonas* has been, perhaps, overemphasised by some workers and minimised by others, but it has become clear that trichomoniasis in the male is a definite clinical condition. If, as appears probable, many patients with trichomonas vaginitis are infected and re-infected by coitus, then study of the infected male becomes a matter of considerable importance; for though human trichomoniasis causes neither death nor acute illness, it is a troublesome infection and until recently was often intractable. The wide variation in reported incidence suggests that a significant proportion of cases of Non-Gonococcal Urethritis may be associated with *T.vaginalis*, but that existing diagnostic methods may be inadequate for the detection of the organisms.

Bauer (1961b) considered that trichomoniasis is the most frequently encountered Venereal Disease in the temperate regions.



### TRANSMISSION

In recent years two symposia on human trichomoniasis have been held, one at Rheims, France, in 1957; and one at Montreal, Canada, in 1959. At both these, the consensus of opinion was that the condition is usually transmitted venereally. And whatever the mode of infection in the female, nearly all authors now agree that infection of the male takes place through sexual intercourse and that trichomonas urethritis in the male should be considered a venereal disease, e.g. Bedoya and Rios (1957a), Keutel (1959), Chappaz (1959), Durel et al. (1960), and Popochristov and Berov (1961).

There are several reasons in favour of sexual intercourse as a mode of spread, and of the pathogenic role of *Trichomonas vaginalis* :

1. Appearance of urethritis in the male after sexual intercourse with a consort harbouring trichomonads.
2. Recurrence of discharge in the male after re-appearance of trichomonas into the female.
3. Examination of consorts of males with trichomonas urethritis shows that the incidence of trichomonas vaginitis is close to 100 per cent.
4. *Trichomonas urethritis* has been produced by local inoculation.

As *T.vaginalis* is a parasite of the human genital tract, sexual intercourse suggests itself as a likely method by which the disease is transmitted. The facts that a not inconsiderable

number of men are found to be infected with the parasite, and that the flagellates are frequently found in the consorts of infected men and women, both lend support to the theory of sexual transmission.

Drummond (1936) described women with recurrent trichomonal vaginitis who were cured only after the parasite had been eradicated from the genital organs of their husbands. By treating both infected partners simultaneously, Bauermeister and Hollinger (1941) and Kucera (1950) were able to prevent recurrences of symptoms in men. Allen and Butler (1946) were able to cure some of their most resistant cases of trichomonal vaginitis only after their husbands had received adequate treatment for their trichomonas infection. Whittington (1951b) described a case in which relapse of the wife's vaginitis corresponded with recrudescences of trichomonas urethritis in the husband.

The frequent association of trichomonal and Neisserian infection in both men and women (King et al., 1936; Liston and Liston, 1939; Liston and Lees, 1940; Allison, 1943) suggests that trichomoniasis like Gonorrhoea, may be transmitted sexually. *T. vaginalis* is found in some cases of Non-Gonococcal Urethritis, and may linger in the prostate for long periods of time, and may be ejected in the semen (Whittington, 1951a). It may also be found in the subpreputial sac and be associated with a balanitis.

Examination of the male consorts of female sufferers has shown a wide variation of positive findings (vide INCIDENCE..Table II, p.37 ), although such statistics may be biased, because those with symptoms rather than those without are more likely to

volunteer for examination. Coutts et al. (1955) reported a case of a husband who developed an acute trichomonal urethritis on his honeymoon. King et al. (1936) found that in a series of 300 female patients with trichomonas vaginitis, the onset of symptoms followed sexual intercourse, usually after an interval of 3 - 10 days. Perez and Blanchard (1957) found 40 per cent of their patients had trichomonas vaginitis and married women gave the greatest incidence, which they considered was due to the fact that unmarried women who expose themselves to infection probably use a chemical contraceptive, which has a disinfectant action. Bedoya and Rios (1958d) found the incidence of trichomonas vaginitis was 73.3 per cent (i.e. higher than average for the population), and in 10 reputed virgins *T.vaginalis* was found; in 7 cases some kind of intercourse was admitted. From this they concluded that trichomoniasis is sexually transmitted, and extra-genital transmission was rare.

Catterall and Nicol (1960a) found 100 per cent infestation in female consorts of male patients with trichomonas urethritis. This figure is well above the approximately 40 per cent infection rate in Venereal Disease Clinic patients as a whole. Five of their patients stated that they were virgins until they had intercourse with their present partners, and that the vaginitis appeared 5 - 28 days after the first intercourse. The authors suggest that these findings support the view that trichomonas infections are usually venereally acquired, and also that patients with trichomonas infections should be referred to V.D. Clinics so that sexual contacts can be traced, examined, and

treated if they are found to be infected.

Teokharov (1960) considered trichomoniasis a venereal disease, and at present the commonest of these diseases and spread by sexual contact. Malinovskii (1960) also believes that *T.vaginalis* is transmitted by sexual intercourse only, as trichomonads are unable to exist outside the human body. Teras et al. (1963) examined 1,284 contacts of 1,157 male and female patients with *T.vaginalis* infections and found 73 per cent were *T.vaginalis* positive. They considered that casual sexual contacts were as important in spreading the disease as more stable sexual partnerships.

Brehm et al. (1961) found that in 20 patients unsuccessfully treated with metronidazole, their male partners harboured the organism; and Döring (1962) considered that late relapses after treatment with metronidazole must be attributed to re-infection from the untreated partner.

Freed (1948) recorded the finding of *T.vaginalis* in 7 homosexuals. Harkness (1950) found *T.vaginalis* on two occasions in the urethral discharge in homosexuals, and in one case they were also present in the preputial sac. Siboulet (1960) in his series had 11 cases of trichomonas infection in males which had been contracted homosexually. Coutts et al. (1955) stated that they had seen a few cases in prepubertal boys, mostly vagabonds living promiscuously, also pederasts fortuitously infected by another, who shortly before had been anally fornicated by a married invert harbouring numerous *T.vaginalis* in the prostate.

Carrier state is obviously important as a focus for the

re-infection of female patients who have themselves been treated for the disease, but where the husbands still harbour the organism. Crowley (1964) considers that man is the cause of the persistence of trichomonal infection in women. He suggested the name *Trichomonas Prostatalis* for the male parasite and *Trichomonorrhoea* for the infection it causes in the vagina.

Sorel (1952) found trichomonads in one clinically normal man whose wife was suffering from trichomonas vaginitis, and postulated the possibility of healthy carriers. Lanceley (1953) found one symptomless "vector" in his series of "normal" married men; and Feo et al. (1956b) found *T.vaginalis* in three asymptomatic patients. Ljahovickij (1960) found two asymptomatic carriers in his series of 190 males with trichomonas infection. Many other authors have similarly reported asymptomatic cases of trichomonas infection (vide CLINICAL FEATURES, p.56). Teokharov (1956) considered that males with little or no symptoms of infection with *T.vaginalis* play a considerable part in the infection of females by the sexual route.

It is difficult not to suspect that some of the ideas about the transmission of *T.vaginalis*, e.g. W.C. seats, towels, etc., are based very largely on an unwillingness to regard so common a condition as a venereal disease. Bourne (1953) asks, if the lavatory seat is a chief factor in *T.vaginalis* infections, why is the infection so seldom seen in virgins, children, and after the menopause.

### NON-VENEREAL TRANSMISSION

Even if it is assumed that infection in the human male is always venereally acquired, it is important to consider also the question of infection of the female. Is this also acquired venereally, or can it be acquired non-venereally and then passed on to the male through sexual intercourse.

One of the main arguments against venereal transmission in the female is based on statistics. Granting that the parasite may be found in, say conservatively, 10 per cent of all females between 15 and 54 years of age, there are some 1,300, 000 women in England and Wales who may be harbouring the organism. On the other hand, in 1963 a total of 24,980 cases of Non-Gonococcal Urethritis were reported from the V.D.Clinics. Even allowing for 40 per cent addition (this is double the figure calculated in an estimate made by the B.M.A. and British Co-operative Clinical Group, 1959) for those cases treated in private practice, and for the increases which may have taken place since, the total number can scarcely exceed 35,000. Most British Venereologists will agree that they cannot find trichomonads in more than approximately 10 per cent of their cases of Non-Gonococcal Urethritis. This gives a theoretical probable total of cases of trichomonas urethritis of 3,500, compared with an estimate of nearly 1.3 million infected women.... a disproportion of nearly 400 : 1. However using statistics to support or disprove theories can be very fallacious. Many European authors in recent years have quoted figures for the incidence of trichomonas urethritis very much higher than British authors, up to 40 and 50

per cent of their cases of Non-Gonococcal Urethritis being due to *T.vaginalis*.

This great difference in the incidence in the male may be due to asymptomatic infection in the male, as obviously a male infected with *T.vaginalis* but having no symptoms will not go for treatment anywhere and therefore the actual infection rate may be very much higher than the figures obtained from clinics. Other factors are inadequacy of diagnostic methods; possible occurrence of a resting stage in the life cycle of the trichomonas, during which stage it cannot be cultured by ordinary means or recognised in smears; the infection may be self-limiting in the male.

There seems to be no doubt that the infection with *T.vaginalis* in the male patient is due to sexual transmission, and it is difficult to contemplate any other method of infection. But in the female it cannot always be due to sexual transmission as many cases are encountered where the male and female partners deny any other consorts, and cases of trichomoniasis have been reported in virgins, true virgins and not only technical virgins.

Schmidt and Kamniker (1926) examined pre- and post-coital vaginal secretions from patients, but failed to find evidence of fresh infection, or of an increase in the number of trichomonads already present, in any of the women after sexual intercourse. If sexual intercourse were the usual method of acquiring the disease, one would expect to find *T.vaginalis* in a larger number of the male consorts of women with trichomoniasis than in fact is the case. Consideration must also be given to

denial of sexual intercourse in patients whose word seems trustworthy. In such cases alternative explanations for infection must be sought.

It is certain that in a large proportion of female cases the infection is acquired sexually, but in others the infection may be by cross-infection; fomites (towels, douche nozzles, specula, etc.), bath water, and the favourite of some authors... the W.C. seat.

Patients have developed trichomonas vaginitis while in hospital (Liston, 1940), and though in many cases this could be due to coital infection prior to admission to hospital (the incubation period in the female is uncertain), simple cross-infection might be possible. The mode of transfer is not yet solved, though the investigations of Whittington (1951b) showed that the flagellates are more resistant to adverse conditions of temperature, etc., than had formerly been believed.

Some authors (notably Mascall, 1954) believed that the vaginal infection arose from the intestinal trichomonad *T.hominis*. This author suggested that the trichomonads enter the body by ingestion, the medium being infected through flies and animals, and dogs and cats are notoriously liable to infestation with trichomonads. Dobell (1934) showed that a single species of trichomonad can live in different hosts, and also in different situations, such as the intestine and vagina. Bland and Rakoff (1937) studied the incidence of trichomonads from the mouth, vagina, and rectum in 200 women. Women harbouring *T.vaginalis* did not show an appreciably higher percentage of buccal and





intestinal trichomonads. Rectal contamination was regarded as an improbable source of vaginal infection in view of the rarity of intestinal trichomoniasis among women with *T.vaginalis* vaginitis.

Hegner (1925) stated that the trichomonad can survive passage through the house fly, and it has also been demonstrated adhering to the feet of flies. Mascall (1954) kept trichomonads alive in milk for 48 hours, and Whittington (1951b) has also demonstrated that they may survive in tap water.

Lanceley (1954) stated that there are no grounds whatsoever for holding that *T.vaginalis* infections in the female may originate from the alimentary tract. This site is not the habitat of *T.vaginalis*, which cannot be implanted successfully in the intestines. Conversely the intestinal trichomonad, *T.hominis* has been found incapable of infecting the vagina. Stabler et al. (1940) inoculated 50 patients intra-vaginally with cultures of *T.hominis* and failed to produce a vaginitis; in these same patients subsequent vaginal inoculations with *T.vaginalis* produced a typical vaginitis.

Some authors have suggested that the infection may arise from domestic animals, but the available evidence is against this. It has been suggested that the infection could be distributed by water during bathing. Bath water is an unlikely source, as it is relatively infrequently shared by adults, and soap is considered to be actively trichomonicidal. Swimming baths have received attention, but the parasite has been shown as unlikely to survive for long in the degree of chlorination found in the

average swimming bath (Weiler, 1938). Paevskij (1957) found that trichomonads stayed motile for 2 to 3 hours in tap and river water at 25 deg. C., but after 6 hours standing the motility was lost. Therefore water transmission of trichomoniasis seems very unlikely.

As far as inanimate objects are concerned; douche nozzles must be very rarely shared, and in good medical practice should never be shared; and douche nozzles, vaginal specula, and rubber gloves are an unlikely source of infection unless an inadequate sterilisation technique is used.

The most likely fomites are towels and W.C. seats. McCullagh (1953) went so far as to say "The main cause of *T.vaginalis* infection is the ordinary lavatory seat", and claimed that the use of the gap toilet seat would reduce the prevalence of the disease by 80 per cent. Whittington (1957) investigated the possibility of lavatory seats being a vehicle for transmitting infection, by placing vaginal material containing trichomonads on lavatory seats and found that the parasites remained alive for up to 45 minutes, and 4 out of 30 patients with trichomonas infection left infective material on a toilet seat after they had used it. Kessel and Thompson (1950) showed that *T.vaginalis* would survive up to 6 hours when vaginal exudate was dried on enamelled blocks of wood.

Whittington (1957) by an ingenious contraption fitted to a water closet seat, showed that half its female users proved to be sitters, and the remainder non-sitters. Therefore it is possible for a non-sitter to deposit urine containing trichomonads

on the seat (where they will survive for 45 minutes) and for a sitter to transfer them to the genitalia. Burgess (1963) investigated the possibility of infection with *T.vaginalis* by contamination by splashing from a W.C. pan. He showed, by dropping faeces substitute into W.C. pans, that droplets of liquid splash as high as the level of the upper surface of the seat; and in one experiment using trichomonas infected urine in the pan, droplets splashed up and onto a sheet of glass laid across the W.C. seat. In a few of these droplets motile trichomonads were found.

However, Patrick (1953), states that in N.E.India and Eastern Pakistan where the Eastern squatting position is universal, the prevalence of trichomonas infection is almost as great as in the United Kingdom.

Evidence regarding towels seems to be more impressive. Burch et al. (1959a) made cultures from a damp cloth which had been used to cleanse the genitalia of 38 women with vaginal trichomoniasis. Of 155 tests made up to 25 hours after use, *T.vaginalis* was cultured in 53 (34.2 %), and of 103 tests made within 3 hours of use, the parasite was cultured in 45 (43.7 %). Brehm et al. (1961) noted that living trichomonads were detected in swabs after 24 hours, which indicated that transmission might occur through articles of clothing. However it seems unlikely that any females are likely to share a wet towel, except within a family, or to share unlaundered underclothing; so I should consider that this method of transmission cannot be of any epidemiological significance.

It might be expected that indirect transmission would be more likely to occur in overcrowded, unhygienic conditions in which personal habits are less fastidious. Some support for this theory might be found if there were differing incidences in ~~1944~~ different social groups. It could account for the known differences between white persons and negroes, the latter frequently being of lower socio-economic status in the community.. .. although the comparative incidence of venereal disease shows that they are also often the more promiscuous. Buxton et al. (1958) found 70 per cent of 221 female prisoners had a trichomonas infection, 15 % of inmates of a mental institution, 6.9 % of obstetric and gynaecological patients, 6.3 % of married and 1.4 % of single employees of an insurance company, and no *T.vaginalis* were found in 157 female undergraduates aged 20 - 22, only 10 of whom were married.

Mascall (1954) suggested that it was possible that the female is the incubator and reservoir of the trichomonads and they only develop to full maturity under certain hormonal stimulation. He disagreed with Feo (1944) who stated "the male is not only responsible for the re-infection of women but is the principle agent of transmission."

## CLINICAL FEATURES

### INCUBATION PERIOD

The incubation period of the infection is dependant on the patient's own statements, and this accounts for the fairly wide variation in incubation periods quoted by various authors, also as many of the patients are promiscuous there is no means of telling which coitus was responsible for the infection with *T.vaginalis*. Periods quoted are: 3 - 8 days (Coutts and Silva-Inzunza, 1952; Bedoya and Rios, 1957a), 1 - 3 weeks (Feo et al. 1956a), 2 - 40 days (Siboulet, 1960), 2 - 210 days (Lanceley, 1953), 6 - 10 days in acute cases (Jira, 1959).

### SIGNS AND SYMPTOMS

Infection in the male may occur alone or in association with gonococcal, fungal, viral, spirochaetal, or other infections, and clinically may be acute, chronic, or latent. Ljahovickij (1960) in his series found only 4.7 per cent of cases were acute, 33.8 per cent asymptomatic, and the remainder subacute or chronic. Jira (1959), on the basis of 185 cases divided male trichomoniasis into two stages; (a) Acute urethral inflammation going on to subacute, chronic or secondary latent stage. (b) Male trichomoniasis first appearing as a latent stage without clinical symptoms or gradually going into sub-chronic stage.

Concomitant Gonorrhoea and trichomoniasis has been observed by many authors; Liston and Lees (1940), Feo et al. (1956a), Hoffman et al. (1961), etc.

There may be no symptoms of infection at all and the

infection is discovered when the patient is examined as a contact of a female with trichomonas infection or when the urine is being examined routinely for other reasons. Many authors reported a considerable proportion of their cases to be symptomless, e.g. Teokharov (1956)... 54.6 %, Whittington (1957)... 19 %, Bedoya and Rios (1957a)... 29 %, Block (1959)... 79 %, Weston and Nicol (1963)... 21 %.

The first objective symptom is usually urethral discharge, which may or may not be preceded by itching around the meatus or slight burning on micturition. Oedema of the prepuce may occur. The discharge may be slight and watery, or milky in appearance, or be abundant and purulent as observed in gonococcal infections; though the purulent type of discharge is seen in only a small proportion of cases. The infection may be associated in a small number of cases with Gonorrhoea and the discharge will then have the character of a gonococcal discharge. The discharge due to a trichomonal infection has no specific or diagnostic appearance. Durel et al. (1954) stated that in 9 of their 35 cases the discharge was frothy, rather similar to that seen in the female; in other cases nothing special was noted. In 1959 the same author emphasised the milky nature of the discharge, although admitting that this is neither constant nor pathognomonic.

Many patients complain of discharge seen chiefly in the morning on rising, at times accompanied by an itchy feeling or some discomfort in the penis and mild dysuria (Feo et al., 1956a). Sorel (1952) also noted morning discharge as a prominent symptom.

Durel et al. (1954) in their series of 35 cases of

T.vaginalis urethritis found 2 cases of Hecht type urethritis, 6 Waelsch type, 15 with a purulent discharge and the remaining 12 had minimal discharge.

The discharge, when present, contains epithelial cells in large numbers, numerous leucocytes, and active motile trichomonads.

#### URETHROSCOPY

There is no specific urethroscopic picture, but Sorel (1952) found stricture in 5 of 17 cases examined. Ljahovickij (1960) made the diagnosis in three cases from material taken from lacunae and Littre's glands, taken under direct vision during urethroscopy.

Ljahovickij and Voskresenskaya (1958) urethroscoped 18 trichomonad carriers and found that in 9 of these the urethra was affected in its entire length, and in 7 cases in the anterior part only. In 2 cases there were no urethral changes. In asymptomatic trichomoniasis the posterior urethra and prostate are affected rather frequently.

#### URINE

Lanceley (1954) stated that one very valuable sign suggests an underlying trichomonad infection ... this was the close association of a heavy trichomonad urethral infection with a urine which was hazy and contained many fine, light, short threads. These threads on microscopical examination are found to consist mainly of epithelial cells. This type of urine

is not present in light infections, but when noted, it is an indication for repeated, and if necessary, prolonged search for the trichomonad. Lydon (1945) similarly noted the significance of this type of urine. Some cases show no urethral discharge, but numerous threads occur in the first portion of an otherwise clear urine. These threads contain pus cells and trichomonads.

The urine in the second glass is usually clear.

#### BALANITIS

The parasite has been found in the preputial sac whether local inflammatory signs are present or not (cf. bovine trichomoniasis) and thus as a potential source of spread of *T.vaginalis* the infected sub-preputial sac is obviously of considerable importance. According to Roth (1944) trichomonas balanitis is not rare in men with a long prepuce. In a group of 40 cases of balanitis studied by Lanceley and McEntegart (1953), in only one case was the parasite not found in some part of the urogenital tract; and Lanceley (1953) quoted the incubation period as varying from 3 - 19 days. Various authors report a fairly high incidence of trichomonas balanitis; see Table VII.

TABLE VII

Author	Year	No. of cases of Balanitis	<i>T.vaginalis</i> positive
Lanceley	1954	40	12.5
Watt & Jennison	1960	30	11.0
Siboulet	1960	15	20.0

Karnaky (1938a) stated that *T.vaginalis* in men caused



itching of the glans penis in some cases, and Mascall (1954) stated that he had seen two cases of severe ulceration of the glans penis and coronal sulcus due to the trichomonas. However Bedoya and Rios (1957a) in a series of husbands of patients treated for trichomonas vaginitis found 78.7 % *T.vaginalis* positive, but no flagellates were found sub-preputially.

#### GENITAL ULCERATION

Ilyin (1955) described an ulcer of the penis due to *T.vaginalis*, and Alfimov (1960) described ulceration of the genitalia in three females and two males; irregular, rounded ulcers, with soft and occasionally undermined edges, and with a greyish yellow film over the base. *T.vaginalis* were numerous in the discharge from the ulcers.

Many authors, including Jirovec and Peter (1954), Bauer (1957), and Keutel (1959) think that *T.vaginalis* infection spreads up the genital tract from the urethra and in untreated cases the posterior urethra and prostate may rapidly be involved. A sensation of weight in the perineum, dysuria, and tenesmus mark the invasion of these structures. The urine will be cloudy in both urine glasses.

According to Lewis and Carroll (1928), and Liston and Lees (1940) other trichomonas manifestations may be seen, such as cystitis or even pyelo-nephritis. Freed (1948) recorded two cases of cystitis and pyelitis due to *T.vaginalis*. Bauer (1957) found in the literature 177 cases of trichomonas cystitis.

Lewis and Carroll (1928) also noted the presence of *T.vaginalis* in the kidney. Coutts et al. (1955) found the parasite in the urine obtained direct from the kidney in two men with chronic pyelitis. Kostic (1959) found *T.vaginalis* in the renal pelvis in 6 (1.7 %) of 364 men with trichomonas infection.

When acute cystitis occurs, pus cells and numerous trichomonads are found in otherwise sterile urine. Coutts (1948) stated he had seen cases of intensive cystitis due to *T.vaginalis*, and cystoscopy showed a congested mucosa, and in some cases a bullous oedema. Nitschke (1936) recorded two cases of bladder infection with *T.vaginalis*. Kostic (1959) found *T.vaginalis* in the bladder in 96 (25.4 %) of 364 men with trichomoniasis, and Capek (1927) observed macroscopic and microscopic haematuria in cases of trichomonas urethritis. Wheatley (1946) and Fieldsend (1946) suggested *T.vaginalis* as a possible "cause" of Abacterial Pyuria. According to Lydon (1945) recurrent attacks simulating pyelitis occur during trichomonas infection in the male. Pyelitis may be attributed to haematogenous origin if we consider the findings of Wagner and Hees (1937).

### PROSTATITIS

Invasion of the prostate is variable and is often a mixed protozoan and bacterial one. *T.vaginalis* invades the prostate and seminal vesicles in a large number of cases, and Bauer (1961b) considered prostatitis to be the most frequent complication of trichomonas urethritis in the male. Many patients with trichomonas prostatitis had never had an urethral discharge,

but most of them had threads in the urine (Coutts et al., 1955). Many authors have reported the presence of trichomonads in the prostatic secretion (see Table VIII). Drummond (1936) used the unusual technique of withdrawing fluid by puncture through the perineum, but the more normal method of prostatic massage is adequate for obtaining a specimen of prostatic fluid.

TABLE VIII

Incidence of Trichomonas Prostatitis

Author	Year	No. of Prostatic specimens examined	No. T.vag. positive
Stuhler	1933	32,000	0.05
Drummond	1936	5	80.0
Cornell & Riba	1936	30	16.6
Allison	1943		1.0
Mascall	1948	200	1.5
Coutts & Siva-Inzunza	1948	12	41.7
Young	1949	171	15.2
Kostic	1954	34	17.7
Turteltaub	1954		20.9
Coutts et al.	1955		40.0
Feo et al.	1956a	31	20.0
Feo & Fetter	1958	24	21.2
Ljahovickij et al.	1958	39	25.0
Bauer	1959a		41.5
Bertrand & Leulier	1959	20	90.0
Ensey	1959	34	61.8
Kostic	1959	364	6.58
Perju	1959	42	84.0
Siboulet	1960	32	25.0
Popochristov & Berov	1961	30	13.0
Feo & Fetter	1961	24	21.2
Nicoletti	1961	9	22.2
Siboulet	1961	109	31.2
Dao	1964	24	45.8

Thompson (1953) quoted a case with chronic abscess of the prostatic ducts due to trichomonas, treated by cautery through a pan-endoscope. Sylvestre et al. (1960) found the prostate to be

infected in 10 of 35 cases of trichomonas urethritis; 7 were cases of trichomonas urethritis and prostatitis, and 3 of trichomonas prostatitis only. Perju (1959) found *T.vaginalis* in the prostate only, in 11 (22 %) of 50 men with trichomoniasis.

Findings in support of diagnosis of trichomonas prostatitis are :

1. No *T.vaginalis* found in the urethral secretion.
2. No *T.vaginalis* in urine or centrifuged deposit.
3. *T.vaginalis* found after prostatic massage, in either the first four or five prostatic drops.
4. The patient complains of prostatic symptoms.
5. After therapy, disappearance of the trichomonads from the prostatic secretion and remission of the symptoms noted.

Ejaculation Praecox may be met with in men with chronic trichomonas prostatitis. In view of the frequent occurrence of *T.vaginalis* in prostatic smears, one would expect to find them at least occasionally in seminal specimens, and in fact many authors have reported finding trichomonads in seminal specimens, (see Table IX.). Coutts et al. (1955) found the parasite in recently ejaculated semen in four cases of recurrent haemo-spermia, and in three of these only moderate numbers of pus cells were seen, in the fourth case they were abundant. Bauer (1957), Keutel (1959), and Kuznik (1960) mention changes in the semen in cases of trichomoniasis. Kolesow (1950) described the immobilisation and phagocytosis of spermatozoa by the trichomonas; and Hoffman et al. (1961) examined seminal fluid from 11 males infected with

*T. vaginalis* and found aspermia in 3, 50 per cent immobile spermatozoa in 4, and no abnormality in 4. Chronic spermato-cystitis has been described and Vargas-Salazar in over 1,000 sperm cell counts in cases of infertility, has found pus cells and *T. vaginalis* in three (Coutts et al., 1953.).

TABLE IX

Incidence of *T. vaginalis* in seminal fluid.

Author	Year	No. of cases examined.	% <i>T. vaginalis</i> positive
Young	1949	151	0.6
Whittington	1951a	26	27.0
Perl et al.	1956	48	58.0
Bertrand & Leulier	1959	16	87.5
Perju	1959	17	35.3
Hoffman et al.	1961	14 consorts	28.5
		73 N.G.U.	9.6
Siboulet	1961	109	13.8

Perl and Ragazzoni (1963) examined seminal fluid from consorts of their patients by asking females to bring a fresh seminal specimen which had been ejaculated directly into a sterile jar, and this was cultured in Kupferberg medium. A second specimen was examined after treatment with Flagyl to ascertain if there had been any effect on spermatozoa --- none was discernible. Unfortunately no figures are given for the number of positives found.

Following on infection of the prostate and seminal vesicles, infection of the epididymis is an obvious further territory for invasion by the trichomonad, and Bauer (1961b)

considered this to be second only to prostatitis in importance as a complication of trichomonas urethritis, occurring in up to 8.5 per cent of cases. Though Acute Epididymitis as a possible complication of *T.vaginalis* infection is still disputed by many authors. Coutts et al. (1955) have seen three cases in which this complication developed during an untreated acute primary infection. They also studied 30 cases of non-specific epididymitis before and after epididymectomy (Saavedra, 1954). In six of these cases the trichomonad was found in the urethra and prostate, and in three of them in scrapings of the removed structure. The incidence quoted by other authors is shown in Table X.

TABLE X

Incidence of Epididymitis in cases of *Trichomonas* infection.

Author	Year	No. of cases.	% <i>T.vaginalis</i> positive
Kostic	1954	34	8.8
Feo et al.	1956a	31	7.0
Feo & Fetter	1958	38	8.0
Siboulet	1960	5	40.0
Popochristov & Berov	1961	38	8.0

Another complication quoted by some authors is stricture of the urethra (Table XI). Riba and Harrison (1940) reported that in 140 stricture cases, *T.vaginalis* was present in 14 (10 %). Hancock in one study of 66 patients suffering from urethral stricture found *T.vaginalis* present in 27 per cent, and in a further series of 17 cases with either stricture or urethral stenosis, *T.vaginalis* was found in 3 (18 %).

TABLE XIIncidence of stricture in cases of *Trichomonas urethritis*.

Author	Year	No. of cases.	% stricture present.
Cornell & Riba	1936	30	16.6
Riba & Harrison	1940	23	61.0
Feo et al.	1956a	31	10.0
Feo & Fetter	1958	38	29.0
Hancock	1959	32	40.6
Catterall & Nicol	1960b	56	9.0

Some authors have quoted systemic complications of trichomonas infections, such as simultaneous arthralgias, arthritis or polyarthrititis during the acute stage. Coutts et al. (1955) have observed these symptoms. Siboulet (1960) found 4 cases of joint involvement and 2 cases of eye involvement in cases of trichomonas infestation.

CHRONIC INFECTION

Kunstmann (1950) considered that some cases of chronic non-specific urethritis were probably due to *T.vaginalis*; and Coutts et al. (1955) claimed that chronic infection is very common and they estimated that 85 per cent of their cases could be classed as chronic.

Most of these patients have a mild or intense urethral discharge, or the sudden appearance of prostatitis, cystitis, or epididymitis. The commonest form of chronic infection is the morning "drop" containing a moderate number of pus cells, large numbers of epithelial cells, and actively motile trichomonads. Other cases present no urethral discharge, but numerous threads

in the first portion of an otherwise clear urine...these threads contain pus cells and trichomonads.

#### PHAGOCYTOSIS

Lejman and Bogdaszewska-Czabanowska (1961) observed phagocytosis of *T.vaginalis* in male patients and in some cases to a significant degree. In one, the number of protozoa engulfed by the leucocytes amounted to 83 per cent of the total parasites observed in the smears. The authors found that while the different stages of phagocytosis could be well observed in stained smears, the best method was by phase contrast techniques. In stained smears the structure of the engulfed parasites at the beginning of the process of phagocytosis showed little or no change. In the later stages, however, the protozoa showed different involutary changes, e.g. homogenisation and partial resorption.

#### TRICHOMONAEMIA

There is a possibility of transient trichomonaemia. During certain acute or chronic localised infections a transient trichomonaemia may be established, as in the post-operative period following prostatectomy.

*T.foetus* has been cultured from the blood of infected cows, and *T.vaginalis* from the blood of infected women (Hees, 1936; Wagner and Hees, 1937; Coutts et al., 1955). The possibility of a generalised infection in human beings would be suggested by a positive serological reaction (Wendelsberger, quoted by Coutts et



al., 1955).

Haemagglutination tests for the presence of *T.vaginalis* antibodies in experimentally infected men gave negative results (Lanceley and McEntegart, 1953), and so far such a test has been positive only in bulls infected with *T.foetus* presenting very severe symptoms.

Trussell et al. (1942) stated that 50 % of women infected with *T.vaginalis* gave positive complement-fixation reactions, and one sixth of the negative cases also gave positive results.

Coutts et al. (1955) believed that in certain circumstances the parasite passed into the blood stream. If this is so, then the parasite may be destroyed, or live and multiply in different organs either as a saprophyte or pathogen. Trichomonads have been found in sections of lung tissue (Marx, quoted by Couterall, 1955), in a subphrenic abscess (Navarro De Alzaga, quoted by Coutts et al., 1955), and in pus from an amoebic abscess of the liver (Gray and Andrews, quoted by Couterall, 1955). Whether the parasites reached these unusual sites by the blood stream or otherwise, is not known.

### DIAGNOSTIC METHODS

For the diagnosis of *Trichomonas vaginalis* infection in the male, specimens are taken from the urethra, sub-preputial sac, prostate, or the urine; and may be examined by any or all of the following methods :-

1. Examination of wet fresh smears by microscopy.
2. Examination of fixed stained smears.
3. Culture of the parasite in artificial media.

If immediate examination is possible, specimens should be taken with a platinum loop. If there will be some delay before examination of the material, a cotton wool swab can be employed, and preferably the swab should be moistened with Ringer's solution containing serum or bacteriological broth (Lanceley, 1954).

Lanceley (1954) considers that examination of urethral discharge is of little value, and that better results are generally obtained by gently curetting the lower part of the urethra with a platinum loop. Weston and Nicol (1963) found that urethral scraping gave 67.7 % positive results, whereas smears of the urethral discharge gave positive results in only 3.25 % of cases.

When taking specimens from the sub-preputial sac, it is important to scrape as widely as possible, paying particular attention to the folds of mucous membrane adjacent to the frenum. According to Lanceley (1954) trichomonads are often recoverable from these folds of mucous membrane, even when all signs of inflammation have disappeared, and scrapes from other areas are

negative. Trussell (1947) also emphasised the importance of examining the sub-preputial sac, calling attention to the fact that bovine trichomoniasis is a venereal disease in which the flagellate finds a suitable environment in the sub-preputial sac of bulls.

The orifices of Tyson's Ducts should also be included in the examination as they may be infected. Prostatic specimens should be obtained in the usual manner. The urine can be examined by taking a freshly passed specimen and centrifuging it. The specimen should be as fresh as possible, as the protozoa become inactive in stale urine. The deposit can then be examined by any or all of the three usual methods. This method of examination can yield good results, and though inferior to examination of urethral scrapings, its routine use should be employed. Lanceley (1954) found the flagellate in freshly passed urine in 18 of 20 cases of known trichomonas urethritis. Young (1949) found trichomonads in 53 of 2,500 centrifuged urine specimens. Sylvestre et al. (1960) found trichomonads in 6 of 35 cases.

Some authorities recommend examination of the seminal fluid as a routine measure.

Karnaky (1938b) advised three smears, one from the urethra before and after prostatic massage, and one from under the prepuce.

It is extremely important to search systematically for the trichomonas in husbands and consorts of infected females, and in males with either urethritis or prostatitis.

### EXAMINATION OF WET FRESH PREPARATIONS

Wet preparations must be examined fresh and within minutes of taking the specimen; either by normal illumination and 1/5" objective or by Dark Ground Illumination, or under the phase contrast microscopy. By this technique, active motile trichomonads can be demonstrated. The recognition of active *T.vaginalis* presents no difficulties, the flagella, especially in motion, are distinct and diagnostic. In general, it is probably unwise to identify *T.vaginalis* without observing flagella or the undulating membrane. The use of safranin in wet preparations has its advocates, but Lanceley (1954) found it of little advantage.

When the specimen contains numerous pus cells it can be diluted with normal saline. An excess of pus cells impedes the free movement of the flagella of the trichomonads. Coutts and Silva-Inzunza (1948) claimed to have demonstrated trichomonads in prostatic fluids previously declared sterile, by diluting the fluid.

A difficulty arises at times in distinguishing rounded motionless specimens of trichomonas from pus cells. Under adverse conditions the trichomonad rapidly becomes rounded and motionless, (Lanceley, 1954; Mascall, 1954), and errors in identification are most common, more especially if one accepts the existence of an encysted or resting phase. It is also possible that mistakes made at this juncture are the cause of conflicting claims for the incidence of male infection. Further errors in diagnosis may be caused by motile spermatozoa attached

to pus or epithelial cells. The resulting movements can be highly suggestive of trichomonad activity.

Coutts et al. (1954) described a vital staining technique which stains the trichomonads, both motile and evolutive forms, but does not stain pus and epithelial cells. In this technique a few drops of 5 % aqueous solution of fluorescein is allowed to penetrate by capillary action under the coverslip of a wet preparation. Within a few seconds all evolutive forms develop a bright emerald green colour which gradually changes to a blue green. Preparations are best examined by Dark Field microscopy. The preparations can be fixed, if required, by introducing 10 % formol under the coverslip 10 minutes after introduction of the fluorescein. The coverslip is then removed and the material spread on the slide in a thin film. The authors claim to have increased their incidence of positive results using this method.

Examination of wet smears has the advantage of speed and simplicity, and can be carried out immediately, giving immediate diagnosis. It may be necessary to search for 20 - 30 minutes, though it is often not possible to spend so much time examining preparations during a busy clinic.

### FIXED STAINED SMEARS

The use of fixed stained smears allows preparations to be examined at leisure. Various staining techniques have been used :

1. Leishmann stain.
2. Methylene Blue stain.
3. Methyl Violet stain.
4. Giemsa stain.
5. Papanicolaou technique.
6. Negri stain.
7. Fluorescent staining.

Stained smears are advocated by many workers such as Schmidt and Kamniker (1926), Liston and Lees (1940), Peter and Jirovec (1950), Fowler (1953), Coutts and Silva-Inzunza (1954), and Harrison (1959).

The drawbacks of cytological methods in detection of these parasites are :-

1. Confusion of parasites with cells or debris.
2. Degree of skill and experience required of the microscopist for correct identification of the parasite.

The advantages, however, are :-

1. Films can be made in the clinic without disturbing the routine and can be examined at leisure.
2. One is not dependant on a single feature, the waving of the flagella, but on other features; distinct outline, granular cytoplasm, oval nucleus.

3. Non-motile forms can be distinguished in stained preparations, and thus increase the number of positive results obtained.

#### LEISHMAN STAIN

This technique was used and advocated by Liston and Lees (1940) in their paper, which was the first on the subject of male trichomoniasis to be published in Great Britain.

The first essential is to use thoroughly clean slides, and Liston and Lees claimed that better fixed preparations can be made by using warm slides. 1cc. of Leishman stain is allowed to act for one minute, then about  $1\frac{1}{2}$  cc. of distilled water is added drop by drop to the stain on the slide and thoroughly mixed. The dilute stain remains on the slide for 10-15 minutes, and is then washed off with a little distilled water. Some fresh distilled water is left in contact with the film for a few minutes to extract any excess of stain. The slide is then allowed to dry in air.

*T. vaginalis* is easily recognised by its light blue colour and oval reddish-purple or magenta nucleus situated towards the anterior end. The flagella and undulating membrane may occasionally stain. The trichomonas can only be confused with an epithelial cell by a careless or inexperienced searcher. The nuclear structure of the trichomonas is very different from that of an epithelial cell, and the parasite is generally larger than a pus cell which, of course, has a multi-lobed nucleus.

### METHYLENE BLUE STAIN

Dock (1896) thought Methylene Blue a better stain than Neutral Red for staining trichomonads, but felt that vital staining should be used or prior fixing in sublimate. Harrison (1959) also advocated staining with Methylene Blue as a more practical and certain method of diagnosis.

The slide is fixed by heat, covered with Loeffler's alkaline methylene blue which has become polychrome with age. The stain is washed off after 45 minutes, mopped and allowed to dry.

Harrison (1959) stated that the diagnostic features are; definite outline, granular cytoplasm, and oval or pear shaped nucleus stained rather less intensely than the nuclei of other cells. The axostyle may also stain.

Mesinev (1960) considered that methylene blue stained smears are the best and most convenient, and demonstration of the flagella is not necessary for diagnosis.

### METHYL VIOLET STAIN

Fowler (1953) recommended staining with Methyl Violet. A drop of 0.5 per cent Methyl Violet 6B in distilled water is placed in the centre of the slide. A loopful of discharge is immersed in the stain and smeared over the slide, allowed to dry, and fixed with heat. Then the preparation is stained with methyl violet for 30 seconds, washed and dried.

The ectoplasm stains lightly and the endoplasm more deeply. The undulating membrane is only occasionally demonstrable



but the nucleus, axostyle, and flagella are not invariably visible but they are seen sufficiently often.

#### GIEMSA STAIN

The May-Grünwald-Giemsa stain was used by Knight and Shelanski (1939), Leca (1951), Sylvestre et al. (1960), Siboulet (1957), Bauer (1959b); Durel et al. (1954) used this technique diluting the Giemsa to 1:10 and increasing the duration of the staining. Jira (1958) considered Giemsa Romanowsky stain better than wet preparations.

The smear is dried in air, and then stained for 3 minutes with a few drops of May-Grünwald stain. An equal volume of distilled water is then added, mixed, and allowed to act for 5 minutes. The slide is then rinsed with distilled water and dried.

The trichomonas stains with a blue cytoplasm, and the nucleus is easily demonstrated flanked by the blepharo-plast.

#### PAPANICOLAOU TECHNIQUE

This technique was used by Wolinska (1959) and Burch et al. (1959b).

The smear is made on a slide and then the slide is placed immediately in a fixative, such as Propyl alcohol. After a minimum of 5 - 15 minutes in the fixative, the slide is passed through successive alcohols 80 %, 70 %, 50 %, and then stained with Harris Haematoxylin for 1 minute. The slide is then washed in water and differentiated in 0.5 % Hydrochloric Acid. After blueing with running tap water, the slide is again passed

through successive alcohols, 50 %, 70 %, 80 %. Counterstaining is performed with OG6 Papanicolaou stain, then after rinsing in two changes of Industrial Spirit, further staining is performed with Papanicolaou stain EA 50 for 1 minute. The slide is finally dehydrated in four changes of Industrial Spirit, cleared in two changes of Xylol, and then mounted.

The trichomonas appears as a greenish-blue structure, oval or round in shape, varying size from 6 - 20 microns. The borders of the parasite are not as clearly defined as those of an epithelial cell. The nucleus is small and dark, and is a helpful guide in differentiating *T.vaginalis* from degenerating pus cells, mucus and debris. The flagella are rarely visible.

#### SELLARS NEGRI STAIN

Allison (1943) used this stain and claimed that the trichomonads were easily identifiable. This author claimed that the technique is tricky, tedious, and time consuming, but worth all it demands. The composition of the stain is :-

Basic Fuchsin	2.4 cc.
Methylene Blue	15 cc.
Methyl Alcohol (acetone free)	25 cc.

The unfixed moist smear is plunged into the stain for a few seconds.

#### FLUORESCENT STAINING

Van Niekerk (1962) recommended acridine-orange staining and fluorescent microscopy as a satisfactory method of detecting fungi, trichomonas, and leptothrix organisms. He claimed that

the living trichomonads stained quite differently from dead ones and are found more frequently in smears stained by this method than on examination of wet smears.

Coutts et al. (1954) described vital staining using 5 per cent fluorescein, and the smears could be fixed with 10 per cent formol.

#### CULTURAL METHODS IN ARTIFICIAL MEDIA

With these protozoa it is impossible to achieve growth on a solid medium. Lynch (1915) was among the first workers to culture *T.vaginalis* using acidified bouillon as a medium. Davis and Colwell (1929) found that human blood serum was essential to active growth of *T.vaginalis*. By 1933 it was apparent that to study the parasite in all its natural forms a pure culture of the protozoan was necessary. Hesseltine (1933) was able to isolate bacteria-free cultures by washing or micro-pipette techniques, but the protozoa would not multiply unless bacteria were added to the medium. He used Locke's solution containing human serum and a small quantity of red blood cells.

Before the advent of antibiotics it was difficult to obtain bacteria-free cultures, but with the use of antibiotics a new field of experiment was opened to workers.

Adler and Pulvertaft (1944) isolated three bacteria-free strains of *T.vaginalis* incorporating in their media, septamide and penicillin. Davis and Grand (1952) stated that Aureomycin Hydrochloride cannot be used in cultures unless buffered to pH 6 to 7 as it has a pH of 3.5 and the acidity will kill the flagellates.

From that date many media have been described for the cultivation of trichomonads, among them being the simplified trypticase serum medium described by Kupferberg et al. (1948) and used by Kean and Day (1954), the C.P.L.M. medium of Johnson et al. (1943 & 1945) which consisted of cysteine hydrochloride, peptone, liver, and maltose. The medium contained a small quantity of agar which made the collection of organisms rather difficult. McEntegart (1952) modified the medium and omitted the agar. Feinberg (1953) in turn modified McEntegart's medium and this modification has proved to be the most successful. This medium consists of :-

Ascorbic Acid	3.7 Gm.
L-cysteine hydrochloride	2.5 Gm.
Proteolysed liver B.P.C.	1.0 Gm.
Glucose	10.0 Gm.
Distilled water	1200 ml.
Bovine serum	160 ml.
Douglas's digest broth, double strength	1000 ml.
N Sodium Hydroxide	to pH 5.8 - 6.0

This medium is sterilised by passing through a Seitz filter and should be used within 14 days. For tube growth 10 ml. amounts are inoculated with 0.5 ml. of an actively growing culture, and incubated at 36 - 37 deg. C.

Feinberg and Whittington (1957) further modified this medium, the new formula being :-

Proteolysed liver	25.0 Gm.
Sodium Chloride	6.5 Gm.
Dextrose	5.0 Gm.
Inactivated horse serum	80 ml.
Distilled water	1000 ml.
Penicillin	1,000,000 units.
Streptomycin	500,000 units.

The solid components are dissolved in the distilled water, the serum added, and the pH adjusted to 6.4 by adding

N/1 Sodium Hydroxide. The mixture is sterilised by Seitz filtration and stored in screw capped bottles in a refrigerator. The authors stated that no deterioration of the antibacterial powers of this culture medium is evident after 3 months storage in a refrigerator at + 4 to + 5 deg.C.

Lanceley (1954) added Methyl or Gentian Violet 1:7,5000 to this medium to control the growth of yeasts. A more effective substance for the control of yeasts nowadays is Nystatin, and Thomas (1964) used this added to the culture medium at a concentration of 1:10,000. Stenton (1957) described a modification of Feinberg's medium which has been used for some years with considerable success.

Specimens taken with a platinum loop can be inoculated directly into the medium, urine specimens are lightly centrifuged, the supernatant removed and the deposit cultured. Growth occurs as a granular deposit at the bottom of the bottle. If a Pasteur pipette is gently introduced and some of the growth carefully removed and examined microscopically, it will be seen that the trichomonads tend to form colonies although there are also numerous free moving protozoa. This clumping of the trichomonads has been described by several workers, including Trussell (1947). It was suggested by Trussell (1947) that this clumping took place around solid particles in the medium and that these were essential to growth. Lanceley (1954) suggests that at least 7 days should be allowed to elapse before cultures are discarded.

McEntegart (1954a) stated that the strains of *T.vaginalis* which the clinician encounters do not survive or grow so well as

the adapted strains which are used for experimental work.

Trussell (1947) reported growth in his culture medium when but one trichomonad was inoculated per ml. Burch et al. (1959b) obtained growth when a single trichomonad was inoculated into a tube of 1 ml. of culture medium by means of a Rees micro-manipulator.

#### SEROLOGICAL TESTS

Reidmüller (1932) tested the sera of a number of women for complement fixing antibody, using *T.foetus* as an antigen. His results were uniformly negative, irrespective of the presence or absence of trichomonas infection. Wendelberger (1936) carried out complement fixation tests with an alcoholic extract of *T.vaginalis* as an antigen, though this culture contained bacterial contaminants. Positive results were obtained in 22 out of 32 sera taken from known cases of trichomoniasis; while 16 sera from apparently healthy women were negative.

A series of 400 sera was examined by Trussell et al. (1942) by a complement-fixation technique using a washed suspension of *T.vaginalis* as the antigen. The test was positive in 52 out of 110 sera from known cases of trichomoniasis (47 %); and of 290 sera from "normal" females tested, 48 were positive (16 %).

A micro-agglutination technique was introduced by Kerr and Robertson (1941) working with *T.foetus*. Trussell (1946) applied a similar technique to human infections with *T.vaginalis*. Having established that the method would give successful results

in experimentally immunised rabbits, 200 human sera were examined, but over 90 per cent of these were recorded as negative and the remainder gave only low titre positive reactions. McEntegart (1952) had previously tested both complement-fixation and micro-agglutination methods for detection of antibody to *T.vaginalis*, but they gave only weak reactions with human serum.

Prompted by the observations of Muniz (1950) who applied haem-agglutination techniques to investigation of Trypanosomiasis, McEntegart (1952) applied the technique of agglutination of sensitised red cells for the detection of circulating antibodies in human trichomoniasis. He tested the sera of 50 "normal" females, and 50 females with *Trichomonas vaginitis*. The results obtained with serum dilutions above 1:10 were 80 % positive in female cases of trichomoniasis, but 6 % positive results were obtained in "normal" male and female patients. Lanceley and McEntegart (1953) attempted to assess the value of this haem-agglutination technique in male patients. But in 5 human male volunteers who were inoculated intra-urethrally with a pure culture of *T.vaginalis*, no antibody could be demonstrated in their sera. And again Lanceley (1954) tested the sera from 174 males, most of whom were suffering from Non-Gonococcal Urethritis; 19 of these, approximately 10 % gave positive reactions by haem-agglutination serological tests. No correlation between these results and the clinical condition of the patients was established.

Hoffman et al. (1963) using trichomonas antigen, performed complement-fixation tests on 275 sera from persons infected with *T.vaginalis*, and obtained 57 % positive results, and

and in 440 sera from persons with trichomonas infection 8.1 % positive results. The authors considered that the method is specific and sensitive.

#### EVALUATION OF DIAGNOSTIC METHODS

It is unlikely that, at the present state of our knowledge, serological tests of any sort will be of any assistance in the diagnosis of male trichomonal infections. The techniques remaining for diagnosis are bacteriological, i.e. microscopy and culture.

For many years the most satisfactory diagnostic technique was the examination of smears, either wet, or fixed and stained. Authors quote varying opinions as to the best technique for microscopical diagnosis of *T.vaginalis* infections. Wet smears were used by Knight and Shelanski (1939), and they considered that, although staining and culturing of material are important in scientific work, the demonstration of the motile protozoon is all that is necessary for clinical diagnosis.

McVay et al. (1951a) used wet smears for diagnosis, and Lanceley (1953) also used wet smears as he claimed, at that time, that stained smears proved unsatisfactory and cultures were not used routinely owing to certain technical difficulties. King et al. (1936) considered that the most satisfactory and accurate method of demonstrating *T.vaginalis* was by examining wet smears, either by Dark Field or ordinary illumination. Liston and Lees (1940), however, recommended smears stained with Leishman stain.

In microscopical diagnosis, the examination of wet smears



is possibly superior to staining of fixed smears unless special experience and skill with the latter are available. Bernstine and Rakoff (1953) considered wet smears superior to cultures, and Barnes et al. (1957) also concluded that careful examination of wet smears was the best method of diagnosis, as in their series only 40 per cent of the smear positive specimens produced positive cultures; and in one case only, where the smear was negative, was a positive culture obtained.

Culture media were not very satisfactory until the real improvement arising from the work of Feinberg and McEntegart, and even with this improvement the culture of *T.vaginalis* demands a delicate and laborious technique. Many workers consider cultures to be most important in diagnosis, and others did not find them to be of any advantage.

Kupferberg et al. (1948) found cultures invaluable and claimed that at least 30 per cent of their cases negative by smears were positive by culture; Kean and Day (1954) also found cultures more reliable than wet preparations for diagnosis of infection. This claim was supported by Whittington (1957), Wolinska (1959), Nicol (1958), and Burch et al. (1959b). But McVay et al. (1951b) stated that "in our hands, cultural methods demonstrated no advantage", and Jira (1958) stated that no significant advantage was offered by culture studies.

Whittington (1957) considered microscopic examination of fresh smears and culture of secretions as diagnostic methods of choice. Perl et al. (1956) advised one wet smear, one stained smear, and one culture. They considered that cultures gave 100%

proof of trichomoniasis.

Lanceley (1954) found that using Feinberg's medium, positive culture results were obtained only with inocula previously found microscopically positive. Further investigations with sub-preputial, prostatic, and vesicular specimens gave similar findings. These findings are no improvement on those published by Magath (1938).

However, the consensus of opinion amongst authors appears to be that cultural techniques for *T.vaginalis* are the most certain method for accurate diagnosis. This has only become so in recent years since the development of the liquid liver medium of Feinberg, and the availability of antibiotics to add to the medium to prevent the growth of bacteria in the medium. However, in culture, the trichomonas does not grow or grows only very slowly for 24 hours, and then grows rapidly in the next 48 hours; after that the rate of growth levels off. This means that no certain diagnosis can be made for at least 24 hours and usually not for a much longer period, as Lanceley (1954) suggests that cultures should not be discarded for at least 7 days.

Wet preparations can be, and in fact must be, examined at once; and if trichomonads are found the diagnosis is available immediately and correct treatment can be given at once.

Cultural techniques are laborious and time consuming, and most bacteriological laboratories are already so overworked that investigations not essential for diagnosis or treatment should be avoided if possible.

### TREATMENT

Until 1959 the treatment of trichomoniasis left a great deal to be desired, as a specific therapeutic agent which could cure the parasitic infection had not been found and none of the various types of therapy commonly used was satisfactory. The treatment often took several months and results obtained were very irregular. The multiplicity of conflicting claims for the ~~success~~ success of the treatment of trichomoniasis may be due to factors concerned with the diagnosis, criteria of cure and the difficulty of establishing a satisfactory series for control.

Of course, research was directed towards treatment of the female, because until the last decade or so, the importance of the diagnosis and treatment of trichomoniasis in the male was not appreciated.

The exhaustive survey by Trussell (1947) showed that only a fraction of the agents used were trichomonocidal in vitro. The possibility was thought to exist therefore, that the disease was self-limiting or that almost any therapy directed to restoring the normal vaginal flora and acidity may occasionally lead to a lasting immunity. This latter theory is, of course, not tenable as there is no immunity, many women have repeated infections of *Trichomonas vaginitis*. Also this theory could not apply in male cases.

Some authors consider that *T.vaginalis* infections are self-limiting in the male, and if left untreated the trichomonad dies off within two to three months, e.g. Karnaky (1938a). Durel et al. (1954) quoted two cases in whom the trichomonas

urethritis appeared to clear spontaneously. Depooter (1959) considered that the urine is toxic for *T.vaginalis* and this explains the spontaneous cure of trichomonas infections in men. This theory is unlikely as trichomonas urethritis in association with trichomonas vaginitis is not uncommon in women, and Durel (1959), by a series of experiments showed that urine has no toxic effect on *T.vaginalis*.

This concept of spontaneous cure has been disputed by many investigators who have followed symptomatic cases for 3 - 5 years (Lydon, 1945; Freed, 1948). Then Lanceley and McEntegart (1953) observed three experimentally infected men and found the organism in the genital tract for 4 - 94 days. Also Whittington (1957) left 19 men, with a *T.vaginalis* infection, without treatment, in order to investigate the possibility of the disease being self-limiting, and the results did not support the view that spontaneous recovery was a common happening.

Block (1959) showed that the presence of the trichomonas in the urethra of the male is not a transient happening, as in 15 of his cases the parasite was still demonstrable long after the infection in the woman had disappeared. M<sub>a</sub>scall (1954) did not agree with the view that trichomonas infection in the male is a trivial affair. He had found it to be, on occasions, very persistent, relapsing and most difficult to eradicate. It is important to treat, if possible, both sexual partners, as there is no doubt that the male is infected through sexual intercourse with an infected female, and most probably the majority of females are infected or re-infected from a male partner.

Catterall and Nicol (1960a) recommended that patients with *T.vaginalis* infections should be referred to V.D. Clinics so that the necessary history of sexual contacts can be obtained. Contacts can be traced, examined, and treated.

There have been a multiplicity of treatments, most of them local applications, recommended for the treatment of trichomonas infection. Bauer (1957) in a review of the literature showed that up to 1943 nearly 100 chemical substances had been or were being used and recommended. By 1955 another 50 could be added to the list. Nearly all these were for local use, especially in women.

Treatment recommended for *Trichomonas urethritis* in the male consisted mainly of local irrigations or instillations. Though Liston and Lees (1940) considered that irrigation of or instillation into the urethra with antiseptics was not curative, and frequently caused harm. Some systemic preparations were used.

### IRRIGATION

Essentially almost every known solution for urethral irrigation has been employed in the treatment of trichomonas urethritis without marked success. Harkness (1950) recommended urethro-vesical irrigations combined with prostatic-vesicular massage. The use of Potassium Permanganate solutions was recommended by Durel et al. (1954), and Catterall (1960). Mercury Oxycyanide solution 1:4,000 to 1:2,000 was recommended by

Grimm (1930), Balkow (1935), and Catterall (1960). Catterall (1960) also recommended Dequalinium Chloride. Oxophenarsine Hydrochloride was used by Angel Garza (1958) in the form of 0.06 Gm. in 0.5 litre of sterile water. Daily irrigations were given for 3 - 5 days and permanent cures were claimed in 24 cases of trichomonas urethritis. Riba and Perry (1929) used Acriflavine Hydrochloride. Drummond (1936) advocated irrigations with 1:8,000 Metaphen combined with injection of 3 cc. of a colloidal silver preparation into the lateral lobes of the prostate through a McCarthy pan-endoscope.

#### INSTILLATIONS

Various solutions and ointments have been recommended for urethral instillation ... Methylene Blue, Mercurochrome, Pentavalent Arsenic, 5 % Argyrol, Acriflavine 1:1,000, Tincture of Merthiolate, Nitro-furan derivatives 1 or 2 per 10,000, Aureomycin 250 mgm. in 5 - 10 ml. of distilled water. Grimmer (1950) observed that under Dark Field examination, after the addition of 1/5th diluted Lugol to the suspension of *T.vaginalis*, the latter stopped moving and their cytoplasm was dissolved. He therefore treated successfully 4 cases with instillations of 5 ml. Lugol.

Conessine Hydrochloride jelly 2.4 % introduced into the urethra after each micturition was recommended by Durel et al. (1954), who occasionally combined it with irrigations with Potassium Permanganate or Mercury Oxycyanide; or alternated the Conessine jelly with Aureomycin ointment 1 %.

Bedoya and Fernandez-Ortega (1958b) recommended treatment for males by inserting specially prepared "pencils" of 2.5 Gm. Stovarsol (3-acetylamino-4-hydroxyphenyl arsonic acid), each 10 cm. in length, into the urethra morning and evening after emptying the bladder.

Knight and Shelanski (1939) used instillations of 0.5 % aqueous solution of Silver Picrate, and claimed all cases were cured. Pelouze (1934) recommended prostatic diathermy combined with instillations of 1:3,000 Acriflavine solution; and claimed that the treatment cleared the trichomonads in a few days.

Le Duc (1955) recommended instillations of Carbarsone 250 mgm. in 1 oz. of sterile water. Andreichuk (1960) used 1 - 4 instillations of 30 % suspension of D.D.T. (Dichlorodiphenyl trichlorethane), after irrigation with 10 - 15 % solution of Sodium Chloride, and claimed that trichomonads disappeared in 30 out of 36 patients treated, and only four patients later relapsed. Artemev (1960) also used D.D.T. in 10 - 20 % suspension and claimed that trichomonads disappeared in 173 of 238 patients treated. He obtained the best results by using a preliminary irrigation of Mercury Oxycyanide (this suggests to me that the effective part of the treatment was the prior irrigation with the Mercury Oxycyanide).

Kupriyanov (1960) treated 28 patients with trichomonas urethritis and 58 with Non-Gonococcal Urethritis with instillations of artificial gastric juice in dilutions of 1:4, 1:3, 1:1, and undiluted, and claimed approximately 50 % cure of trichomoniasis, and 30 % cure of Non-Gonococcal Urethritis.

Sorel (1952) stated that the results of local instillations in the treatment of trichomonas urethritis were not encouraging.

#### SYSTEMIC THERAPY

An ideal trichomonocidal agent should exert its effect systemically, which would ensure its even distribution throughout the body and reach all mucous membranes and infected glands (Plentl et al., 1956). Many preparations have been tried by the oral route with conflicting reports as to the success of the therapy.

Liston and Lees (1940) believed that rendering the urine alkaline inhibited trichomonads and relieved symptoms, and might produce cure. Weston and Nicol (1963) claimed 33 % of their cases were cured by Mist. Pot. Cit.. Strain (1945) favoured Calcium Mandelate by mouth and claimed cure of 7 out of 8 male cases of trichomoniasis. Freed (1948) recommended the use of Gentian Violet or Amanol to disinfect the urinary tract via the blood stream.

Allen and Baum (1943) found little or no effect from treatment with systemic administration of Urotropin (hexamethylene tetramine) and Sodium Acid Phosphate. A series of compounds of well established protozoocidal, though not necessarily trichomonocidal, properties were tested by Schwartzwelder et al. (1954). No significant effect on vaginal trichomoniasis was detected. Carpenter (1952) had given an optimistic report on systemic Chloroquine therapy, but this was



not confirmed by Schwartzwelder et al. (1954).

#### ARSENICAL PREPARATIONS

Arsenical preparations were an obvious choice for oral therapy in the male in view of their comparative effectiveness in local treatment in the female. Rodecurt (1936b) claimed 75 per cent cure with Spirocid, an oral preparation containing acetyl-amino-hydroxy-phenyl-arsonic acid. Pattyson (1937), on the other hand, found this preparation had no effect at all when administered by mouth; and reported similar failure with Fowler's solution (Liq. Arsenicalis), sodium arsenate, Mapharsen, Carbarsone (p-carbamino-phenyl-arsonic acid). Trussell (1947) stated that none of these agents are appreciably trichomonocidal in vitro. Ackermann (1935) found Salvarsan to be of no value. Durel et al. (1954) however used Acetarsol 1 gm. per day, four times a week for four weeks either alone or alternating with Aureomycin; and Coutts and Silva-Inzunza (1948) cured 5 cases of trichomonas prostatitis with arsenical therapy. Alfimov (1960) obtained rapid healing of trichomonal ulcers with Acetarsone (Spirocid).

Patiāla and Vara suggested the use of tetracyclines, Jirovec (1957) recommended Thiolutin, and Chappaz (1951) advocated Gramicidin and Endomycin, but the results proved unsatisfactory.

### AUREOMYCIN

Collins et al. (1948) and Herrell et al. (1949) showed that after oral administration of Aureomycin, large amounts are absorbed into the general circulation and excreted in the urine. McVay et al. (1951a) therefore treated 11 males with trichomonas infection with Aureomycin. In all cases symptoms disappeared within one week and in 9 of the patients there was no evidence of relapse. Dosage used was 750 mgm. four times daily for three days and then 500 mgm. four times a day for four days. If the prostate was involved the dosage was 3 Gm. daily for three days, and then 2 Gm. daily for seven days. Davis and Grand (1952) believed that the clinical results of treatment with Aureomycin might be due to the acidity of the product rather than a special effect of Aureomycin.

### NITROFURANTOIN

Ensey (1959) treated 12 males with Nitrofurantoin (Furadantin), each one had trichomonas prostatitis, and good results were claimed for the treatment.

### TRICHOMYCIN

Trichomycin was isolated from *Streptomyces hachijoensis* in Japan by Hosaya (1954). Reports from Japan indicated that it was effective against trichomonads both in vitro and in vivo. It was also claimed to be effective against *Candida Albicans*.

Its in vitro activity was accepted by Bertrand (1957), Catterall (1957), and others; but its in vivo activity did not

give the expected results. Catterall and Nicol (1957), Bedoya and Fernandez-Ortega (1958a), and Bauer (1959b) found it of no therapeutic value. However Durel et al. and Siboulet obtained one cure, and Sylvestre et al. (1958) obtained two cures with trichomycin. According to Catterall and Nicol (1957) trichomycin does not confer trichomonocidal activity on the serum and urine.

ACINITRAZOLE (2-acetyl-amido-5-nitrothiazole) (Tritheon, Trichorad)

The in vitro and in vivo trichomonocidal action of Acinitrazole was discovered by Cuckler et al. (1955). Its in vitro activity was confirmed by Cavier et al. (1957) and Catterall (1957). Plentl et al. (1956) and Perl et al. (1956) claimed it was effective clinically, but Catterall and Nicol (1957), and Barnes et al. (1957) obtained unsatisfactory results. Feo and Fetter (1958) concluded that there was no evidence that the drug was of any therapeutic value. Willcox (1957) and Dunlop et al. (1958) found it completely ineffective, as did Bedoya and Fernandez-Ortega (1958a), who also suggested that previous reports of success with the drug were due to inadequate tests of cure.

I, personally, found this drug completely ineffective in the treatment of trichomoniasis in the female.

METRONIDAZOLE (Flagyl)

Horie (1956) described the trichomonocidal properties, in vitro, of 2-nitro-imidazole, discovered by Nakamura (1955). Cosar and Julou (1959) confirmed the activity of this substance and proceeded to study another derivative, more active and less toxic; 1-(2-hydroxyethyl)-2-methyl-5-nitro-imidazole (Flagyl).

Early results with Metronidazole (Flagyl) indicated that it was the first oral remedy effective against human genital trichomoniasis, and it promised to be a notable advance in the treatment of this common condition. Many authors have reported the efficacy of this drug in the treatment of trichomoniasis in both the male and female, e.g. Sylvestre and Gallai (1960), King (1960), Nicol et al. (1960), Feo and Fetter (1961), Siboulet (1961).

The standard course of treatment with Flagyl is 200 mgm. (one tablet) three times daily for seven days. This is sufficient to cure almost all trichomonal infections. If necessary, the course can be repeated with the same dose, or the dose increased to 400 mgm. three times a day for seven days. It may be necessary to use the higher dose in cases where a complication, such as prostatitis, is present. Rodin et al. (1960) found that the response to the drug was not so satisfactory where the trichomonas urethritis was complicated by stricture, littritis, or prostatitis. Concomitant local therapy is not necessary in either the male or the female.

No serious toxic effects of the drug have been reported. There have been minor gastro-intestinal disturbances in some

cases (Siboulet, 1961; Durel et al., 1960); less often patients have experienced dizziness, drowsiness, headache (Feo and Fetter, 1961), depression (Rodin et al., 1960), agitation, insomnia, bad taste in the mouth, and brown furring of the tongue (Siboulet, 1961); rashes, pruritus (Siboulet, 1961). Disturbances severe enough to necessitate cessation of treatment have been extremely rare. Many authors report no toxic effects noted in their series, e.g. Nicol et al. (1960).

In laboratory animals metronidazole showed a wide range of toxic effects, including testicular damage with disturbance of spermatogenesis, and neurological disorders such as ataxia and tremor. The testicular damage resulted from very high dosage in rats and did not develop on repetition of the experiment with lower but still high dosage. The neurological disorders resulted from overdosage in dogs and did not occur in other animals. Bauer (1961a) found that Flagyl had no effect on the motility of spermatozoa in vitro.

Some concern has been expressed about the possibility of blood disorders, since the drug is an imidazole bearing the nitro group, and similar drugs have caused these toxic effects. Studies of the effects on the white blood cells have been few, but they have given no cause for alarm. Serial white blood cell counts have usually shown no significant changes, and no cases of serious blood dyscrasias have been reported. King (1960) performed serial total and differential white blood cell counts on 41 cases, mainly during the first two weeks after treatment was started, and in 3 cases the count fell below 1500,

but all rose to above normal lower limit at the next count. Csonka (1963) reported similar findings.

According to Durel et al. (1960) Flagyl renders the serum trichomonocidal at a dilution of 1:10 for three hours at least, and the urine trichomonocidal for over four hours at dilutions of 1:100 to 1:1,000. This is the only compound which imparts a high trichomonocidal activity to the serum and the urine. Kane et al. (1961a) found urinary excretion of Flagyl was variable and there was no evidence of accumulation of the drug in the serum.

The remarkable effectiveness of orally administered Flagyl focusses attention on the small number of patients who have failed to respond to this therapy. These failures may be due to :-

1. Resistance of certain "strains" of *T.vaginalis* to Flagyl.

Robinson (1962) reported a strain of trichomonas resistant to metronidazole. Squires and McFadzean (1962) investigated cultures from 26 patients failing to respond to metronidazole, and found that strain sensitivity to metronidazole was in the same range as the stock strains isolated before introduction of the drug.

2. Inability of the host to absorb a sufficient amount of the drug.

Kane et al. (1961b) investigated 8 female patients who failed to clear on a standard course of Flagyl, and demonstrated that 4 of the patients showed significantly lower serum levels,

and concluded that poor absorption of the compound may be responsible for a percentage of failures with the drug.

3. The survival of a few flagellates for long enough to initiate a relapse when treatment is stopped.

Some authors, (Lydon, 1945; Wenyon, 1926; Marshall, 1954; Siebenthal, 1945; Schinozuka, 1955; Zinser, 1941; and Lanceley, 1954), believe that there is a "resting" or encysted phase in the trichomonad life cycle into which the organism passes when subjected to an unsuitable environment.

4. Re-infection from the untreated male or female partner.

Both partners should be treated if possible, and it is considered useless to treat a female patient without also treating her male partner (Perl et al., 1956; Bedoya and Rios, 1957a; Bauer, 1961b). Catterall (1963) stated that treatment must be considered for male contacts with urethritis in whom *T.vaginalis* is not found, and for males who are shown to have infected their female contacts.

Catterall and Nicol (1960b) recommended that sexual intercourse should not take place until the parasite has been eradicated from the genital tract of both partners. Young routinely advised the use of a condom by the husbands of patients with a trichomonas vaginitis.

Durel et al. (1960) investigated the effect of metronidazole on Non-Gonococcal urethritis in which *T.vaginalis* was not found, and reported that their results were practically negative, whereas

negative, whereas Sylvestre et al. (1960) reported success in all of five cases in which *T.vaginalis* was not found, although the female consorts of these patients were known to harbour the parasite (this suggests that in fact these males were probably infected with the trichomonad but it was not demonstrated). Willcox and Rosedale (1962) treated 70 cases of Non-Gonococcal Urethritis in whom *T.vaginalis* had been excluded by wet smears and/or culture from the urethra and prostate, and considered that metronidazole was not effective in cases of N G U in which trichomonads are not found.

Relapse or apparent failure of treatment with Flagyl must be regarded with suspicion, as many of these are probably re-infections; and the probability is increased if the sexual partner is not treated. Brehm et al. (1961) found that in 20 patients unsuccessfully treated with Flagyl, the male partners harboured the organism. Doring (1962) considered that late relapses after treatment with Flagyl must be attributed to re-infection from the untreated partner.

Williams (1962) concluded that his 99.2 % cure rate in infected women was due to his concomitant administration of metronidazole to the husband as well as to the wife, although the husband was rarely investigated for the presence of trichomonas. Sikat et al. (1962) reported recurrence of the wife's infection if the husband was not treated with metronidazole at the same time; and in their series all the husbands were treated empirically, none of them were examined.

Kemkes (1962) believed that the discovery of metro-



nidazole and the simultaneous treatment of husband and wife have virtually eliminated trichomoniasis as a clinical problem. Rosenblum (1962) in co-operation with 12 Obstetricians and Gynaecologists, came to the conclusion that oral metronidazole given to both husband and wife was such an effective measure that it would eventually eradicate the problem. This, I think, is wishful thinking as similar pronouncements were made in the past regarding Gonorrhoea when penicillin was discovered, and the incidence of Gonorrhoea is now the highest for 20 years and is still rising.

### PRESENT INVESTIGATION

This investigation into the incidence of trichomoniasis of the male uro-genital tract was carried out during the years of 1963 and 1964, at the Special Treatment Centre, Royal Infirmary, Derby. All male patients attending who complained of urethral discharge, some men with Balanitis, and some asymptomatic patients, were examined for the presence of *Trichomonas vaginalis*. In addition, sexual partners of women with *T.vaginalis* vaginitis, and female partners of male patients with Non-Gonococcal Urethritis, were examined; if they were known, and could be persuaded to attend for examination.

All women attending the clinic during the same period, in whom full genital examination was performed, had routine vaginal smears for the presence of *T.vaginalis*. This is my normal practice, but because of the investigation of male patients, care was taken to ensure that all female patients were examined for infestation with *T.vaginalis*.

This investigation involved the examination for the presence of *T.vaginalis* of a total of 525 men and 460 women. From the male patients a total of 1,152 wet preparations were examined by Dark Field microscopy, and 2,739 stained smears; and from female patients a total of 617 wet preparations.

All patients had a detailed history taken, with particular note of the date of the last coitus, and duration of symptoms prior to attendance at the clinic. Physical examination with particular reference to the genito-urinary tract was performed at the first visit. All patients with a urethral discharge, however minimal,

minimal, had routine smears of the urethral discharge stained with Gram's Stain, and cultures for the gonococcus were taken either by plating a loopful of discharge directly onto a blood agar plate or by the use of a charcoal swab and Stuart's Transport Medium (Moffet et al., 1948).

Next, the necessary smears for investigation for the presence of trichomonads were taken. Material was taken by means of a platinum loop from the urethral mucous membrane by the urethral scrape technique as recommended by Lanceley (1954). This technique is easier to perform when the discharge is minimal, as the loop becomes contaminated with discharge, if this is profuse. In addition, the prostatic secretion, after prostatic massage, of 250 of the men was examined.

Those patients who had no urethral discharge had smears made of urethral scrapes, of any deposit from the centrifuged urine specimen (the urine deposit from patients with urethral discharge was also examined), and of prostatic secretion. Patients with Balanitis had smears taken from the sub-preputial sac, paying particular attention to the fossa navicularis (Lanceley and McEntegart, 1953).

Specimens taken from the male patients consisted of three smears :

1. One wet preparation examined immediately by Dark Field illumination. If the discharge was thick the preparation was diluted with Normal Saline.
2. One smear allowed to dry in air and later stained by Leishman stain.
3. One smear placed in

3. One smear placed in a fixative of Propyl Alcohol for a minimum of 15 minutes, and later stained by the Papanicolaou technique.

Cultures for *T.vaginalis* were not taken as the technique is laborious and time consuming. Previous attempts to culture *T.vaginalis* in a commercially prepared medium, using inocula from proved cases of *Trichomonas vaginitis* in women, were totally unsuccessful.

The standard two glass urine test was performed, and the first urine specimen was lightly centrifuged and three smears of any deposit made similarly to the urethral specimens. All patients had blood specimens taken for routine serological testing for Syphilis.

In female patients specimens were taken with a platinum loop from the posterior vaginal fornix through a vaginal speculum, and a wet preparation examined by Dark Field illumination.

In those patients with urethritis the Gram stained smear immediately classified them into two groups --- those with Gonorrhoea and those with Non-Gonococcal Urethritis. Those patients with Gonorrhoea had prostatic smears taken approximately one week after routine treatment of the Gonorrhoea, as I consider it inadvisable to massage the prostate while there is an acute urethritis present.

All the male patients with urethritis were asked to persuade their wives and/or consorts to attend for examination. Unfortunately no great success was achieved in this investigation

of consorts, as the majority of the patients were promiscuous and consorts were unknown to them.

The 525 male patients examined could be classified as follows :

1. 250 men with Non-Gonococcal Urethritis.
2. 218 men with Gonorrhoea.
3. 14 men with Balanitis.
4. 43 men who were asymptomatic.

#### INCIDENCE

In the two year period of the investigation, I found the total incidence of Trichomoniasis in the 525 males examined to be 8.19 per cent of all patients, and 10.8 per cent of cases of Non-Gonococcal Urethritis, and 4.58 per cent of cases of Gonorrhoea. Detailed figures of the incidence obtained in this series of patients are shown in Table XIII (p.105).

The finding of 10.8 per cent incidence of Trichomoniasis in cases of Non-Gonococcal Urethritis corresponds fairly well with the incidence reported by many authors in the United Kingdom. It is, of course, lower than that reported by many European and American authors. The incidence of combined infection with Gonorrhoea and *T.vaginalis* I have found to be 4.58 per cent of all cases of Gonorrhoea, which is higher than that quoted by some authors, though it corresponds with the figures of Hoffman et al. (1961), and is lower than those of Bogacheva (1958).

13.95 per cent of the asymptomatic patients were found to have trichomoniasis, and coincidentally, this group of patients

TABLE XIIIncidence of Trichomoniasis in 525 patients investigated.

Diagnosis.		No. of cases	T.vaginalis positive	Per Cent.
N.G.U	White	188	16	8.5
	Negro	62	11	17.7
	Total	250	27	10.8
Gonorrhoea	White	145	4	2.76
	Negro	73	6	8.22
	Total	218	10	4.58
Asymptomatic	White	34	3	8.8
	Negro	9	3	33.3
	Total	43	6	13.95
Balanitis	White	13	0	0
	Negro	1	0	0
	Total	14	0	0
Total	White	280	24	8.57
	Negro	145	19	13.1
	Total	525	43	8.19

Detailed analysis of T.vaginalis positive cases p. 114 - 117.

Illustrative case reports p. 118 - 122.

with trichomoniasis formed 13.95 per cent of the total cases of trichomoniasis found. This high incidence in asymptomatic patients may be artificially high due to the fact that this group of patients consisted mainly of men who were requested to attend for examination as they were husbands or consorts of women with trichomonas vaginitis.

Table XII shows that the incidence of trichomoniasis is higher in Negro patients, a finding which agrees with the results obtained by many authors (e.g. Feo et al., 1956a., Whittington, 1957., Weston and Nicol, 1963).

The 468 patients with urethritis can be classified as shown in Table XIII (p.107). This table shows that the incidence is higher in married men among the white patients, and equal in married and single Negro patients. This finding does not agree with the figures given by Feo et al. (1956a), though in their series the Negro patients were greatly in the majority. Also in my series, although the Negro patients are shown as married, very few of them were living with their wives as they are all immigrants, and the majority of them have not yet brought their wives to this country.

#### EXAMINATION OF CONSORTS

Examination of wives and/or consorts of patients with urethritis, and particularly of any patients with trichomoniasis demonstrated an incidence of trichomonas vaginitis as shown in Table XIV (p.108).

TABLE XIII

Classification of 468 patients with urethral discharge.

Race	Diagnosis	Cases No.	%	Age groups (years)			Married	Single
				Under 20	20-29	30-39		
White	Gonococcal	145	43.5	12	61	42	66	79
	Trichomonas negative positive	141 4	2.76	0	1	2	4	0
	Non-Gonococcal	188	56.5	13	81	49	112	76
	Trichomonas negative positive	172 16	8.5	1	9	3	11	5
Negro	Gonococcal	73	54.0	1	33	27	43	30
	Trichomonas negative positive	67 6	8.22	0	1	4	3	3
	Non-Gonococcal	62	46.0	1	35	22	28	34
	Trichomonas negative positive	51 11	17.7	0	4	5	5	6



TABLE XIVExamination of wives/consorts of male patients.

Male patients.	No. of consorts examined	T.vaginalis positive	Per cent.
N.G.U	29	18	62.0
Gonorrhoea	38	13	34.2
T.vaginalis positive	24	22	91.7

In addition the examination of husbands and/or consorts of female patients with *Trichomonas vaginitis* showed a high incidence of infection with *T.vaginalis*. 37 males were examined and 21 (56.7 %) were found infected. This figure corresponds closely with the figures obtained by Watt and Jennison (1960).

During the years 1963 and 1964 a total of 79 female patients were treated for a Gonococcal infection, and of these 79 patients, 23 (29.1 %) also had a trichomonas infection; and of a total of 177 female patients requiring treatment for a non-gonococcal condition 97 (54.8 %) had a trichomonas vaginitis. Therefore of a total of 256 female patients with genital conditions requiring treatment, 120 (46.86 %) suffered from a trichomonas vaginitis. In addition a total of 204 female patients were examined fully and no conditions requiring treatment found. Thus out of a total of 460 females receiving a full examination, trichomonas infestation was found in 120 (26 %).

### CLINICAL FEATURES

It was impossible to assess the probable incubation period for the development of the trichomonas urethritis. Most of the patients were promiscuous and had coitus several times in previous weeks, both marital and extra-marital. Even though a patient's wife or consort were examined and found to have trichomonas vaginitis, it would be impossible to decide which act of coitus had been responsible for causing infection in the male. If one accepts the most recently admitted act of coitus as responsible the incubation period varied from one week to two months, and the symptoms when present appeared within 5 - 10 days of the patient's attendance at the clinic.

The urethral discharge in those cases with urethritis presented no typical characteristics, and varied from slight mucoid discharge to the frankly purulent discharge due to associated Gonorrhoea. In two cases the discharge was white and "frothy" in appearance, as described by Durel et al., (1954), and Durel (1959). No special characteristics of the first urine specimen were noted.

In 25 of the 43 cases (58.1 %) the trichomonad was found in urethral specimens, and in 19 cases (44.2 %) this was the only site of infection (see Table XV, p.110). In asymptomatic cases the trichomonad was found in the urethral scrape in only one case, and the prostate was the only site of demonstration of the trichomonad in 66.6 % in these asymptomatic cases.

In the 10 cases of concomitant Gonorrhoea and trichomoniasis the trichomonad was demonstrated in the urethral smears at

initial examination in two cases only, in a further two cases trichomonads were demonstrated in the urethra one week after routine treatment for the Gonorrhoea had been given. The remaining cases were diagnosed by demonstration of the trichomonads in the prostatic secretion or urine specimen deposit, after routine treatment of the Gonorrhoea.

TABLE XV

Site of demonstration of *T.vaginalis* in male patients.

Diagnosis	N.G.U.		Gonorrhoea.		Asymptomatic	
Site.	No. of cases	Per cent.	No. of cases	Per cent.	No. of cases	Per cent
Urethra	20	64.0	4	40.0	1	16.7
Prostate	9	33.3	7	70.0	5	83.3
Urine	11	40.7	3	30.0	1	16.6
Urethra only	11	40.7	2	20.0	1	16.6
Prostate only	4	14.8	5	50.0	4	66.6
Urine only	2	7.4	0		0	

The results of diagnostic methods used are shown in Table XVI (p.111). It is notable that in 10 of the 43 cases (23.2 %) trichomonads were found in stained smears only, and not in wet preparations. But in only one case were the trichomonads found in a wet preparation and not in the stained smears from the same patient.

TABLE XVIResults of Diagnostic techniques used in men T.V. positive.

Diagnosis	Diagnosis made by :					
	Wet smears only		Stained smears only		Wet + stained smears	
	No. of cases	Per cent.	No of. cases	Per cent.	No. of cases	Per cent.
N.G.U.	1	3.7	6	22.2	20	74.1
Gonorrhoea	0		3	30.0	7	70.0
Asymptomatic	0		1	16.7	5	83.3
Total	1	3.7	10	23.2	32	73.1

Wet preparations were examined immediately, and as far as possible, 5 - 10 minutes were spent examining the preparations, though it was often not possible to spend longer than 5 minutes examining the wet preparations as I did not have the time for prolonged examination during a busy clinic as I am working without any assistance.

Stained preparations were examined at leisure, and more time could be devoted to the examination of these slides. All stained smears were examined as soon as possible after the patient's first attendance and always before the next attendance.

#### TREATMENT

Metronidazole (Flagyl) was used routinely for treatment of all male patients in whom trichomonads were demonstrated in the

uro-genital tract. The standard course of treatment, 200 mgm three times daily for seven days, was given. After treatment all patients were kept under surveillance for a period of three months, and smears taken by urethral scrape and prostatic massage were examined regularly. However, very few patients completed full surveillance, as the default rate was very high and also many patients were re-exposed to various infections.

As far as possible, both sexual partners were treated if *T.vaginalis* was found in either partner. Two husbands of women with trichomonas vaginitis, who could not or would not attend the clinic for examination, were supplied with a course of Flagyl via the wife. A further 11 male consorts who were examined, but in whom *T.vaginalis* could not be found, were also treated with Flagyl. Of these 11 men, 4 had Non-Gonococcal Urethritis, 6 had Gonorrhoea (as did their wives or consorts), and one was asymptomatic.

A further 56 men with Non-Gonococcal Urethritis in whom *T.vaginalis* could not be found, and whose consorts were unknown, were also treated with a course of Flagyl. These patients had all had previous treatment with Demethylchlortetracycline (Ledermycin), 150 mgm 6 hrly for five days, and all had some evidence of persistence of urethritis, e.g. slight discharge, pus threads in the urine, or complained of a discharge in the mornings on rising; and no trichomonads could be found in the specimens from the urethra and prostate. Of this total of 60 patients with Non-Gonococcal Urethritis treated with Flagyl 22 (36.6 %) were relieved of their symptoms, and remained clinically clear for a period of at least six weeks surveillance.

The results of treatment of 20 patients (33 %) were unknown as they all defaulted from surveillance within 10 - 14 days of starting treatment with Flagyl, though one might be entitled to assume from this default that their symptoms had cleared. The remaining 18 patients were not relieved at all by the course of treatment with Flagyl.

As Willcox and Rosedale (1962) and Durel et al. (1960) found that Flagyl was ineffective in the treatment of non-trichomonal Non-Gonococcal Urethritis, it would be reasonable to suggest that most, if not all, of the 22 patients with Non-Gonococcal Urethritis apparently cured by Flagyl were, in fact, cases of uro-genital trichomoniasis in whom the trichomonad was not found.

In only one patient were toxic effects noted. This male patient complained of vomiting while taking the Flagyl tablets, but he persevered and completed the course of tablets.

Patients showing evidence of T.vaginalis infection.

No.	Diag.	Age	M or S	Race	Type of Disch.	Urine	1	2	T.V. present in U.	U.	Pr.	Consorts, comments, etc.
1	Gon	31	M	Negro	P	H	C	-	-	+	+	Wife GC+ T.V.+ Consort unknown. T.V. found after Gon. treated.
2	Gon	39	M	White	P	H	VSH	-	-	+	+	Wife GC+ T.V. +. Consort GC+ T.V.+ T.V. found after Gon. treated.
3	GOn	27	M	White	P	H	C	+	0	0	0	Consort unknown.
4	Gon	47	M	Negro	P	H	C	+	-	-	-	T.V. in prostate after Gon. treated.
5	Gon	28	S	Negro	Nil	Sht	C	+	+	-	-	Urine deposit GC+. Defaulted.
6	Gon	31	M	White	P	H	C	-	+	+	+	Wife GC+ T.V.+
7	Gon	38	M	Negro	P	H	C	-	-	+	+	T.V. found after Gon. treated.
8	Gon	39	S	Negro	P	H	C	+	+	+	+	Consort unknown.
9	Gon	53	M	White	P	H	C	-	-	+	+	Wife GC+ T.V.+
10	Gon	45	S	Negro	P	SH	C	-	-	+	+	Urethritis persisted after treatment for Gon. T.V. found in prostate.
11	NGU	42	M	Negro	Muc.	C	C	+	0	0	0	Consort unknown.
12	NGU	25	S	Negro	MP	H	C	+	0	0	0	Consort unknown.

No.	Diag.	Age	M or S	Race	Type of Disch.	Urine	T.V. present in U.	Pr.	Consorts, comments, etc.		
						1	2	Ur.			
13	NGU	25	M	White	Muc.	H	C	+	0	0	Consort unknown. Wife would not attend.
14	NGU	27	M	White	MP	Sht	C	+	0	0	Consort unknown. Wife would not attend.
15	NGU	25	M	White	SMP	Sht	C	+	-	-	Marital coitus only. Wife would not attend.
16	NGU	29	S	White	VSMP	SH	C	+	-	0	Consort unknown.
17	NGU	30	S	Negro	SMP	Sht	C	+	-	+	Consort unknown.
18	NGU	19	S	White	SMP	SH	C	-	+	0	Consort unknown.
19	NGU	37	M	White	VSMP	Ct	C	+	-	-	Wife T.V. +
20	NGU	30	S	Negro	VSMP	Ct	C	+	+	0	Unknown consort
21	NGU	43	M	White	MP	SH	SH	+	0	0	Marital coitus only. Wife T.V. +
22	NGU	28	S	White	MP	H	C	+	+	-	Consort unknown
23	NGU	40	M	White	VSMP	Ct	C	+	+	0	Discharge and haemorrhage 18 months before in Japan.
24	NGU	23	M	White	MP	H	C	+	0	0	Wife had vaginal discharge, but would not attend.
25	NGU	21	S	White	SMP	Ct	C	+	+	+	Consort T.V. +



No.	Diag.	Age	M or S	Race	Type of Disch.	Urine 1 2	T.V. present in Ur. U. Pr.	Consorts, comments, etc.
26	NGU	42	S	Negro	Muc.	Ct C	- - +	Divorced. Ex-wife T.V +
27	NGU	36	M	White	MP	H O	- - +	Wife T.V +
28	NGU	25	M	Negro	SMP	Ct C	- - +	Wife T.V +
29	NGU	26	M	Negro	VSMP	Ct C	+ + -	Wife T.V +
30	NGU	34	M	Negro	SMP	H H	+ O O	Wife T.V +
31	NGU	29	M	White	MP	Sht C	- + -	Consort unknown. Wife not examined.
32	NGU	36	M	White	SMP	Ct C	+ + O	Wife T.V +
33	NGU	21	S	White	SMP	Ct C	+ O O	Consort T.V +
34	NGU	33	S	Negro	SMP	Ct C	- - +	Consort T.V +
35	NGU	27	S	Negro	SMP	Ct C	+ + +	Consort unknown.
36	NGU	35	M	Negro	SMP	Ct C	+ + +	Wife pregnant, would not attend.
37	NGU	53	M	White	Muc.	VSht C	- + +	Wife T.V + Monilia +
38	NIL	23	M	Negro	Nil	C C	+ - -	Wife T.V +
39	NIL	20	M	White	Nil	C C	- - +	Wife T.V +

No.	Diag.	Age	M or S	Race	Type of Disch.	Urine	T.V. present in Ur. Pr.	Consorts, comments, etc.
40	NIL	27	M	White	Nil	C C C	- - +	Wife T.V. +
41	NIL	30	S	Negro	Nil	Ct C	- + +	Unknown consort one year ago.
42	NIL	27	M	White	Nil	C C C	0 0 +	Wife T.V. +
43	NIL	27	M	White	Nil	C C C	- - +	Wife T.V. +

Discharge.

P = Purulent  
 MP = Muco-purulent  
 SMP = Slight muco-purulent  
 VSMP = Very slight muco-purulent  
 Muc = Mucoid

Urine

C = Clear  
 H = Hazy  
 SH = Slight haze  
 t = threads in urine

Ur. = Urethra  
 U. = Urine deposit  
 Pr. = Prostatic smear.

T.V = T.vaginalis      + = present      - = not present      0 = not examined for T.V.

Diag. (Diagnosis) = diagnosis at initial examination.

ILLUSTRATIVE CASESCASE No. 1.

Negro. Married. Aged 31. Extra-marital coitus three weeks previously (consort unknown), marital coitus one week previously. Discharge for four days, no dysuria. Urethral smears contained Gonococci but no trichomonads. Wife was then examined and found to have Gonorrhoea and Trichomonas vaginitis. Prostatic smears from the patient one week after routine treatment for Gonorrhoea showed the presence of trichomonads in both the wet and stained smears.

CASE No. 2.

White. Married. Aged 39. Extra-marital coitus one month previously, and marital three weeks previously. Discharge for one week. Gonococci, but not trichomonads, present in urethral smears. Wife and consort examined and both found to have Gonorrhoea and Trichomonas vaginitis. Patient examined again one week after routine treatment for Gonorrhoea, and trichomonads were found in stained smears of prostatic secretion.

CASE No. 5.

Negro. Single. Aged 28. Stated that last coitus was two months ago, but that discharge had been present for only three days, and had been treated by his G.P. with Penicillin tablets orally. On examination, no discharge was present, but the first specimen of urine was hazy with pus. Smear from the urethral scrape contained trichomonads, and in the deposit from the centri-

-fused urine both Gonococci and trichomonads were demonstrated.

CASE No. 8.

Negro. Single. Aged 39. Last admitted coitus five weeks before. Discharge present for two days. Gonococci present in smears, but no trichomonads in wet smears, though trichomonads were found in the stained smears from the urethra and urine deposit. After treatment of the Gonorrhoea and before treatment was given for the trichomoniasis, trichomonads were also demonstrated in the prostatic smears.

CASE No. 9.

White. Married. Aged 53. Extra-marital coitus two weeks previously, and marital five days previously. Acute Gonorrhoea, no trichomonads in urethral smears or in urine deposit. Wife found to have Gonorrhoea and Trichomonas vaginitis. Prostatic smears from patient after treatment of Gonorrhoea demonstrated trichomonads in both wet and stained smears.

CASE No. 11.

Negro. Aged 42. Married (wife in Jamaica). Extra-marital coitus four weeks previously, but stated that urethral discharge had been present for three months. Only a slight mucoid discharge present with a clear urine. Treated with Sulphonamides and the condition apparently cleared but recurred after three weeks. No further coitus admitted. A few epithelial cells and trichomonads present in the wet smears.

CASE No. 12.

Negro. Single. Aged 25. Under treatment for multiple anterior urethral strictures. Gonorrhoea twice and Non-Gonococcal Urethritis once previously. Frequent recent coitus with different unknown consorts. Mucopurulent urethral discharge present for two days. Pus cells, epithelial cells and trichomonads in wet smear from the urethra.

CASE No. 21.

White. Married. Aged 43. Marital intercourse only. Thick white mucopurulent discharge present for three days. Trichomonads were present in wet and stained urethral smears. Wife examined and found to have Trichomonas vaginitis. This patient had been under treatment for several years for chronic recurrent Non-Gonococcal Urethritis without the true cause being recognised.

CASE No. 23.

White. Married. Aged 40. Marital coitus only. History of "infection of bladder" with urethral discharge and haemorrhage 18 months ago in Japan. Referred for examination as his wife was under ~~treatment for~~ Trichomonas vaginitis. Very slight mucopurulent discharge present, with clear urine with a few threads in it. Trichomonads were present in wet and stained smears from the urethra, and in the urine deposit.

CASE No. 36.

Negro. Married. Aged 35. Marital coitus only. Wife pregnant and would not attend for examination. Discharge present for three days. The urethral discharge was white, muco-purulent, and "frothy" in appearance. Urine clear with threads in the first specimen. Trichomonads were present in urethral and prostatic smears, and in the urine deposit.

CASE No. 37.

White. Married. Aged 53. Marital coitus only. Slight mucoid discharge, which had been present for two weeks. First urine specimen slightly hazy. Previous history of Latent Syphilis and Non-Gonococcal Urethritis. No trichomonads found in urethral specimens, but present in stained smears (though not the wet smears) of the centrifuged urine deposit and prostatic secretion. Wife found to have Trichomonas vaginitis.

CASE No. 39

White. Married. Aged 20. Referred for examination by his wife, who had attended for treatment of a vaginal discharge, and was found to be suffering from Trichomonas vaginitis. No urethral discharge and urines clear, but trichomonads found in wet and stained smears of prostatic secretion.

CASE No. 41.

Negro. Single. Aged 30. Last coitus one year previously. Discharge in the mornings for several weeks. There was no urethral discharge, urine clear, and the prostate felt normal, but large numbers of trichomonads and epithelial cells were present in the wet and stained smears of the prostatic secretion.

CASE No. 43.

White. Married. Aged 27. Extra-marital coitus one year ago. Complained of irritation in the urethra. No urethral discharge present and urine clear. No trichomonads found in the smears of urethral scrape, but the parasite was present in stained smears of the prostatic secretion. His wife was examined and found to have *Trichomonas vaginitis*.

### DISCUSSION

Trichomoniasis of the male uro-genital tract has become recognised universally as a clinical entity and a condition of some importance only in the last 10 - 15 years. Before that, the condition was recognised, and in fact was described 80 years ago, but scant attention was paid to the subject and reports were few and far between.

I think there is no doubt that *Trichomonas vaginalis* is a pathogenic organism, and the work of Christian et al. (1963) with tissue cultures has proved this. The experimental work of Lanceley and McEntegart (1953) showed that clinical infection could be produced by inoculation of cultures of the organism into the uro-genital tract of human male volunteers. All previous theories that the *T.vaginalis* is a saprophyte, or only produces its clinical effects in symbiosis with another organism (e.g. a streptococcus), can be discarded; similarly the theory that the trichomonad came from the bowel is no longer tenable.

There seems to be no doubt that in the male the transmission of the infection or infestation is sexual in nature (i.e. venereal). It is impossible to visualise any other method by which the trichomonad could be introduced into the male urethra. The finding of a high incidence of trichomoniasis in the sexual partners of patients with trichomoniasis, e.g. in my series 91.7 % trichomonas vaginitis in female consorts of male patients with trichomoniasis, and 56.7 % incidence of trichomoniasis in male consorts of women with trichomonas vaginitis; and similar figures reported by many authors; is strong evidence in



favour of venereal transmission.

The main argument against venereal transmission is a statistical one, that, assuming the estimated incidence of trichomoniasis in the female population to be 10 %, and in the highly selected group of patients attending a Venereal Diseases Clinic I found the incidence among all women examined was 26 %, the incidence in males should be similar if not higher. The incidence of Gonorrhoea reported by Clinics shows a male to female ratio of approximately 3 or 4 : 1, as one female can infect several men, and it is accepted that signs and symptoms of Gonorrhoea in the female can be overlooked very easily by the infected female. The estimated ratio of male to female trichomoniasis in the United Kingdom is in the region of 400 : 1. However, statistical arguments can be very misleading, and I consider that in some men the infection can be self-limiting, mainly for anatomical and physiological reasons. The site of the infection in the male is the urethra, which is irrigated frequently with urine of varying pH, as compared with the vagina of the female which is virtually a closed pocket of infection. In addition a large number of infected males are asymptomatic, and the infection is only discovered when they are examined as consorts of women with trichomonas vaginitis, or for some other reason. In my series 14 % of the cases of male trichomoniasis were asymptomatic, and various authors have reported the incidence of asymptomatic infection varying from 14.3 % (Catterall, 1960) to 79 % (Block, 1959). I agree with the statement of Chappez and Chatelier (1951) that "trichomonas vaginitis may be transmitted

to a woman by a clinically healthy man, the penis merely being a vehicle for the parasite.", though I do not agree with the implication in this statement that the penis is merely a vehicle and does not suffer any ill effects itself.

I believe that the infection in the female is mainly by sexual transmission, but that non-venereal transmission can occur in some cases. It is difficult to explain the infection of children, virgins, and hospital in-patients by any other method. The work of Whittington (1957) and Burgess (1963) suggests a possible method of non-venereal infection from the W.C. seat, though contamination by infected fomites is, I think, a more likely method; particularly damp towels.

The greatest resistance to acceptance of trichomoniasis as a venereal infection has come from Gynaecologists and General Practitioners who do not appreciate that their patients are unlikely to give them accurate sexual histories. The Venereologist, with his special training and experience, is much more likely to obtain an accurate history of sexual intercourse. I find that I very often obtain an entirely different sexual history from that which had been given to the patient's General Practitioner. There is still a stigma attached to the term "venereal disease", though it only means sexually transmitted disease.

The lack of attention paid to the subject of trichomoniasis in the male has, possibly, been due to a lack of appreciation of the importance of this infection in the male as an important factor in the epidemiology of *T.vaginalis*; and due

to the failure to accept trichomonas vaginitis in the female as a condition which in most cases has been acquired sexually.

The lack of satisfactory cultural techniques until recent years has hampered research, and has possibly made it likely for some cases of infection to be undiagnosed and therefore produced figures for the incidence of infection of the male which are lower than the true incidence. But I do not think that cultural techniques would produce very much higher figures, provided that skilled and experienced examination of smears is carried out conscientiously. In most reported series the author or authors have used wet smears for diagnosis, sometimes with a combination of stained smears and/or cultures; and in very few series would the number of cases of trichomoniasis been much smaller without the use of cultures. In the series of Barnes et al. (1957) and Weston and Nicol (1963), in only one case in each series was the organism detected in culture after it had failed to be discovered in wet smears. Ljahovickij (1960) obtained 83.3 % positive results with wet smears, and only 77.7 % positive results with cultures.

For clinical use, as opposed to experimental work, I consider microscopic techniques are adequate for diagnosis, and cultures for the trichomonad to be a useful adjunct if the Pathological Laboratory is prepared to do the work involved. In view of the tremendous pressure of work on these laboratories nowadays it is unlikely that much assistance could be provided for culture of the trichomonads. After all, culture for the gonococcus is not considered essential for diagnosis, except in

cases with medico-legal complications, though cultures are very valuable confirmation of diagnosis and in the very occasional case, usually female, culture may be positive where the gonococcus has not been found in the direct smear. According to the figures quoted by various authors, only 2 - 4 % of *T.vaginalis* infections in males would be missed by failure to take cultures, and in some series no case would have been missed by the omission of cultures, particularly when a combination of wet preparations and stained smears has been used, e.g. Wolinska (1959) found 70 % of his cases positive by wet smear, and 90% by stained smears, and Perju (1959) 73 % positive by wet smear and 80 % positive by stained smear.

It has been suggested that cultural techniques would help to produce a positive diagnosis in those cases in which the trichomonad is in a "resting" non-motile state and thus would not be easily identified in wet smears unless the fluorescent staining method of Coutts et al. (1954) were used. Many authors, e.g. Coutts and Silva-Inzunza (1954), Petcherskiy (1951), Keutel (1958), and others consider that non-motile forms exist, and Fruhwald (1957), Keutel (1958), Mesinev (1960) considered that these non-motile forms predominate in males. Malinovskii (1960) stated that motile forms were only seen in the late stages of the infection in men. I believe that these non-motile forms do exist, as in several of my female patients who appeared clinically to be suffering from trichomonas vaginitis, no trichomonads could be found in the wet smears but the report on smears taken for staining with the Papanicolaou technique for detection of early

Cancer of the Cervix, stated that trichomonas vaginalis was present. In 10 (23 %) of the male patients in my series, trichomonads were detected in the stained smears only. This would suggest that in stained smears the non-motile forms, which cannot be detected in wet smears, are stained normally and exhibit the characteristic appearance of trichomonads.

It could be suggested that this finding is pure coincidence and that the specimen taken for examination by wet smear, fortuitously did not contain any trichomonads and the specimen taken for staining did contain trichomonads. This argument would appear to me to stretch the long arm of coincidence rather far.

A more valid argument would be that if more time had been spent examining the wet smears in these cases, the trichomonad would have been found. I did spend as long as possible, within the limits of a busy clinic, examining wet smears; and the criticism would not account for those cases in which trichomonads were not found in the wet smears after searching for a reasonable time, but were found in the stained smears after a very short search.

I recommend Leishman staining of smears as the method of choice. I tried Giemsa, Methylene Blue, Methyl Violet, and Leishman stains, and found the Leishman stain to be the most satisfactory and simplest in use. I used two staining methods, Leishman stain and Papanicolaou technique. The Papanicolaou technique is time consuming and I consider that the trichomonads are not easy to recognise unless the microscopist has considerable

experience. I, personally, spent much time studying several hundred known *T.vaginalis* positive Papanicolaou smears from the Index of Diagnosis files in our Pathological Laboratory before I felt competent to recognise *T.vaginalis* in Papanicolaou stained smears.

Leishman staining, carried out as described by Liston and Lees (1940), is a simple technique and the trichomonads stain distinctively. A photomicrograph is attached to the cover of this thesis, and this is a photograph of a Leishman stained smear from one of my male patients with trichomonas urethritis. This shows, within the limits of colour reproduction, a clump of trichomonads which are quite distinctive. The appearance is quite different to that of the pus cells, the pus cells staining magenta and the trichomonad bluish, also the large multilobed nucleus of the pus cell is obvious. Differentiation from epithelial cells is easy as the trichomonad has a small dense nucleus situated at one end, and the epithelial cell has a large central nucleus. I consider that trichomonads can be differentiated after a small amount of experience of examining the smears. Occasionally the flagella stain, sometimes the axoneme, and these points also help to identify the trichomonad.

Infection of the male uro-genital tract is not confined to the urethra. I have myself demonstrated the trichomonad in urine specimens and in the prostatic secretion. Many authors have shown that there is a high incidence of trichomonas prostatitis (Table VIII, p.62). Also a high incidence of the presence of *T.vaginalis* in the seminal fluid was shown by

Whittington (1951a), Bertrand and Leulier (1959), and others (Table IX, p.64). Epididymitis due to *T.vaginalis* has also been described by several authors.

The finding of *T.vaginalis* in 56.7 % of a series of male patients whose female consorts suffered from trichomonas vaginitis, and the incidence of 91.7 % trichomonas vaginitis in female consorts of men with trichomoniasis, shows the desirability of examining both sexual partners for evidence of trichomoniasis. Recurrent attacks of trichomonas vaginitis in a female patient should raise suspicion of re-infection rather than relapse, and attempts should be made to persuade the husband or consort to attend for examination. Most authors are now agreed that examination and treatment of both sexual partners are the ideal for epidemiological control of trichomoniasis.

It is, however, often not possible to examine or treat both sexual partners, in particular the consorts of those female patients who attend a V.D. Clinic for treatment. The male patient is often infected by an unknown consort, and the married female patient may not wish her husband (who may have been infected by her, or may have infected her) to know that she has attended a V.D. Clinic for treatment, whether she has been exposed to extra-marital infection or not.

Though by experience and training the Venereologist is best suited to examine males for evidence of *T.vaginalis* infection, this examination is usually only possible in the case of unmarried patients. Even examining consorts at a place other than a V.D. Clinic, e.g. in Gynaecological Department or Women's Hospital, can raise

can raise suspicion of venereal disease, as in the mind of the lay public even in the better educated examination of, or disease of the genital area must be connected with venereal disease.

Watt and Jennison (1960) found that, in spite of repeated re-assurances, some of the husbands they examined still harboured doubts about the possibility of venereal disease.

It is, however, desirable that both sexual partners should be treated for trichomoniasis, and the ideal would be collaboration between the Gynaecologist and Venereologist, possibly by arranging for the Venereologist to examine husbands and consorts of women with *T.vaginalis* vaginitis who attend the Gynaecological Clinic; and if possible the examination should be carried out elsewhere than in the V.D. Clinic. I have made some progress towards such an arrangement in my area. If, however, examination of consorts should not be possible, and the consort is known, I consider that Flagyl should be supplied to the patient to give to the consort and the consort should be persuaded to take the tablets, even though no symptoms are present. Treatment without diagnosis is, I admit, not very satisfactory, but in view of the efficacy of Flagyl and its minimal side effects, its use for epidemiological control of trichomoniasis is an important consideration.

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### CONCLUSIONS

1. In approximately 10 % of all males with Non-Gonococcal Urethritis, the *Trichomonas vaginalis* is the causative organism. The incidence is appreciably higher in Negro patients than in white. This contrasts with the incidence of 54.8 % trichomoniasis in female patients with Non-gonococcal conditions.
2. *Trichomonas* infestation is found in association with the gonococcus in 4.5 % of male patients suffering from Gonorrhoea, and again the incidence is higher in the Negro than in the white patient; This incidence contrasts with the female where *T.vaginalis* is found in association with the gonococcus in 29 % of cases.
3. Approximately 14 % of cases of *Trichomonas* infection of the males are asymptomatic. In some other cases symptoms were minimal and therefore infection can pass unnoticed. This can be considered to be akin to a "carrier" state and therefore important epidemiologically.
4. Female consorts of male patients with trichomoniasis were found to be 91 % infected with *T.vaginalis*, and female consorts of male patients with Non-Gonococcal Urethritis were found to have 62 % incidence of trichomonas vaginitis, and it is considered that *T.vaginalis* can be the cause of a Non-Gonococcal Urethritis in men, even though the trichomonad

cannot be demonstrated.

5. It is considered that a combination of a wet preparation and stained smear, preferably stained Leishman stain, are together adequate as a practical method easily used in a busy clinic, for the diagnosis of trichomonas infection of the male uro-genital tract. These preparations should be made from specimens obtained by urethral scrape, from the deposit in centrifuged urine, and from prostatic secretion where obtainable.
6. Trichomoniasis of the male uro-genital tract is a sexually acquired disease and can therefore be classed as a venereal disease.
7. Metronidazole (Flagyl) is an extremely effective systemic therapy for trichomoniasis, and is in fact the only suitable and effective therapy for male patients. To ensure fully effective treatment it is necessary to treat both sexual partners.

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